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ENVIRONMENTAL RELATIONSHIPS IN A SEED-BORNE DISEASE OF BARLEY CAUSED BY *HELMINTHOSPORIUM SATIVUM* PAMMEL, KING, AND BAKKE[1]

BY H. W. MEAD[2]

Abstract

The experiments described in this article show that the greatest seedling injury occurs under conditions that are unfavourable to the host, i.e., high temperature and excessive moisture or low temperature and scant moisture; that the greatest recovery takes place at 15 to 18° C. in moist soil. They further show that packing and fertilization of the soil increase the amount of infection, though fertilization may increase the dry weight of the seedlings; that the microflora of the soil has little influence upon the seed-borne parasite; that reduction of the oxygen content of a nutrient solution and of soil from 21% to 10% depresses the growth of the seedlings and the amount of infection; that the addition of 1% of carbon dioxide to the atmosphere of soil increases the amount of seedling infection. From these results the conclusion is drawn that barley that is infected with *H. sativum* should be sown in cool, moist, well aerated soil.

Introduction

In a previous article (13) it was shown that *Helminthosporium sativum* Pammel, King, and Bakke may parasitize barley spikelets. When infected mature kernels were sown, the resulting seedlings showed evidence of blighting, malformation, stunting, and lesioning, according to the conditions of growth. This article deals with experimental work conducted in the greenhouse and in field plots for the purpose of studying the effects of certain environmental factors on the disease, as found in two samples of barley, one from Manitoba, the other from New Brunswick.

Environmental Factors

A. Soil Microflora

There is little information on the effect of the microflora of the soil on the development of seedling blight or root rot arising from naturally infected seed. If the soil microflora prevents or appreciably inhibits seedling injury which usually arises from infected seed, it would be of considerable economic importance. Christensen (2) found that there were no differences in germination or amount of seedling injury when barley seed naturally infected with

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Helminthosporium or *Fusarium* was planted in sterilized and non-sterilized soil. Similarly, the addition of *Trichoderma lignorum* (Tode) Hanz and several other fungi and bacteria to naturally infected barley seed did not inhibit or delay the parasitic action of the seed-borne parasites. Hynes (10) showed that under some conditions biological antagonism was not of importance in limiting injury from *H. sativum*. Henry and Campbell (9) studied the effect of sowing flax seed that was naturally infected with *Polyspora Lini* Laff. in sterilized and natural soil. A consistent and marked reduction in disease was evident in the natural soil as compared with the sterilized soil. Indeed, the severity of infection was reduced more by antibiotic action than by seed treatment. However, in preliminary tests with wheat naturally infected with bunt fungi, there was no reduction in infection in natural soil as compared with sterilized soil.

Experiment I

Greenhouse tests were conducted with the New Brunswick sample of barley. It was grown in sterilized and natural soil to determine any differences in infection due to soil microflora. The seed was sown in loam soil in six-inch pots. One-half of the pots contained sterilized soil, the other half natural soil. Similarly, untreated seed and Ceresan-treated seed were used. The pots were arranged in a four by four Latin square on the greenhouse bench and were kept moist by surface watering. The results of two tests are summarized in Table I.

TABLE I

EFFECT OF SOIL STERILIZATION ON EMERGENCE AND INCIDENCE OF DISEASE IN BARLEY FROM SEED INFECTED WITH *H. sativum*

Seed	Soil	Emergence, %	Disease rating, %
Healthy	Natural	98.5 ± 1.6	2.2 ± 1.7
Healthy	Sterilized	98.0 ± 1.4	2.9 ± 1.4
Diseased	Natural	67.2 ± 9.9	46.1 ± 12.7
Diseased	Sterilized	71.5 ± 14.4	42.9 ± 10.6

The data were examined statistically by Fisher's analysis of variance method as outlined by Goulden (6). The differences in the amount of disease in sterile and natural soil were not significant.

B. Temperature and Moisture

Dosdall (3) studied the temperature relations of *Helminthosporium sativum*. She found that mycelial development occurred over a wide range, 1° to 37° C., with the best growth near 28° C. Germ tubes penetrated coleoptile and leaf tissue from 12° to 34° C. but severe infection and rapid development occurred only between 22° and 30° C. Above 30° C. the development of lesions seemed to be checked. Jones, Johnson, and Dickson (11) found that the cereals, wheat and barley, grew best at relatively low temperatures, 12° to 16° C.

Dosdall (3) found that the percentage and severity of infection in barley seedlings, as determined by plant growth, increased as the amount of soil moisture was increased. Her results also indicate that seedling barley plants suffer most from root infection in soil containing maximum or minimum extremes of moisture. McKinney (12), working with wheat, found that high soil temperatures (28° to 32° C.) and high soil moisture content (55 to 65% moisture holding capacity) favoured infection. When the soil temperature was high, high soil moisture content was most favourable for infection, while at low temperatures, parasitic attack was greater at lower soil moistures. Extremely low soil moistures were not favourable to infection at any temperature, and at extremely high and low temperatures the moisture curves for infection were irregular.

Experiment II

Six combined soil temperature and moisture tests were conducted in soil in the greenhouse. In Tests 1 and 2 the soil was sterilized; in Tests 3, 4, 5, and 6 it was not sterilized. Two moisture levels were used, these being 30 to 35% (low) and 50 to 55% (high) and they were maintained by regular weighings of the containers and surface watering. In Tests 1 and 2, the sample grown in Manitoba was sown and the New Brunswick sample was used in Tests 3, 4, 5, and 6. In Tests 3 and 4 the air and soil temperatures were controlled alike; in the other tests the air temperature was that of the greenhouse. The results of the tests are given in Table II.

The results obtained from the two samples of infected barley were quite different. This might be expected, because different varieties, possibly infected with different strains of the fungus were grown in sterilized and natural soil respectively. In Tests 1 and 2, where the Manitoba sample was used, there was little infection at 12° C., in either the high or low moisture series. The amount of infection increased as the temperature was raised to 21° C., but it was affected very little by increase in moisture. The strain of *H. sativum* present on this sample of seed was not particularly virulent. It caused very little seedling blight and only moderate lesioning. The strain of *H. sativum* on the New Brunswick sample was very virulent. It caused considerable seedling blight and from moderate to severe lesioning at 12°, 18°, and 25° C. whether the moisture content of the soil was low or high. Moisture was a limiting factor, however, at 32° C.; the greatest amount of blighting and lesioning occurred in the high moisture series. Because the soil in Tests 3, 4, 5, and 6 was not sterilized, control tests using disease-free susceptible barley were conducted, and only a trace of lesioning occurred on a few plants. It is evident then that the injury done to the seedlings was caused by the seed-borne fungus.

Experiment III

Field tests were conducted in 1941 with the New Brunswick sample of barley for the purpose of studying the effect of early, medium, and late sowing on emergence. The seed was sown in rows six inches apart in four blocks with

TABLE II

SUMMARY OF EMERGENCE AND LESIONING IN BARLEY FROM SEED INFECTED WITH *H. sativum* GROWN UNDER CONTROLLED CONDITIONS

Test	Soil moisture	12° C.		16° C.		18° C.		21° C.		25° C.		32° C.	
		Emerged, %	Lesioned, %	Emerged, %	Lesioned, %	Emerged, %	Lesioned, %	Emerged, %	Lesioned, %	Emerged, %	Lesioned, %	Emerged, %	Lesioned, %
1	Low	98	6	94	26			98	48				
	High	100	2	92	30			96	34				
2	Low	94	8	100	26			94	40				
	High	100	6	98	36			90	38				
3	Low	90	50							100	80	100	20
	High	90	50							80	80	90	80
4	Low	80	20							90	90	100	20
	High	100	30							90	90	100	30
5	Low	86	60			82	62			84	60	52[1]	12
	High	86	68			80	70			90	78	92	40
6	Low	86	76			80	66			84	56	94	20
	High	86	72			80	78			92	88	84	76

[1] *Reduction in emergence caused by uneven moisture distribution.*

four replicates in each block. Rows containing some of the same seed treated with Ceresan were included in the tests. The difference in emergence between these rows and those containing untreated seed was ascribed to blighting by the seed-borne fungus, *H. sativum*. The results of the field tests are given in Table III.

TABLE III

THE EFFECT OF DATE OF SEEDING UPON THE EMERGENCE OF BARLEY FROM SEED INFECTED WITH *H. sativum*

Date of seeding	Emergence		Necessary difference*
	Untreated, %	Treated, %	
April 26	56.7	88.9	14.9
May 30	70.0	93.3	11.8
June 30	43.6	85.6	24.3

* *Necessary difference, 5% level of significance, between untreated and treated series.*

In the field the most severe blighting occurred during the week following the third date of seeding when the mean daily soil temperature was 20.1° C. This temperature is higher than the optimum for barley seedlings but lower than the optimum for the fungus. There was more blighting following the first date of seeding than following the second. It appears probable that the soil conditions for host development were most favourable during the period following the second date of seeding and that blighting was less severe because of that condition.

In general, the greenhouse and field experiments indicated that when a virulent strain of *H. sativum* was present on barley seed, it infected the seedlings throughout a wide range of temperature and moisture. Where there was just sufficient moisture for strong germination, the fungus was active at low and moderate temperatures, but was inhibited at very high temperature (above 30° C.). An increase in soil moisture made little difference at low and moderate temperatures but increased damage from infection at high temperature. The results suggest that a crop of barley would suffer least damage from *H. sativum*, when borne on the seed, if the seed were sown when soil conditions, moisture and temperature, favoured the barley plants.

C. Firmness of Seed Bed

Experiment IV

Observations made while removing infected barley seedlings from soil indicated that the shoots of certain plants were weak, and being unable to push through the soil, died in a short time. Other severely infected plants were sufficiently strong to emerge, produce one or two leaves, and survive for two or three weeks. A greenhouse test was planned to study the effect of packing the soil on emergence, stunting, and blighting in the case of barley

seed infected with a virulent strain of *H. sativum*. Untreated and treated seed was sown two inches deep in moist loam soil in six-inch pots. The soil in one-half of the pots was pressed firmly after the seed was sown. There were four replicates of each treatment. The pots were set in a four by four Latin square arrangement on the greenhouse bench, and were kept moist by surface watering. The greenhouse temperature range was from 16° to 26° C., with a mean of about 22° C. The data are summarized in Table IV and an analysis of the emergence is given in Table V.

TABLE IV

EFFECT OF PACKING SOIL ON EMERGENCE AND DISEASE IN BARLEY FROM HEALTHY SEED, AND SEED INFECTED WITH *H. sativum*

Seed	Soil	Test	Emergence, %	Stunted, %	Blighted, %
Healthy	Unpacked	1	98	1	2
		2	98	1	2
		3	96	2	4
Healthy	Packed	1	99	0	1
		2	97	0	3
		3	100	2	0
Diseased	Unpacked	1	80	21	20
		2	84	10	16
		3	75	10	25
Diseased	Packed	1	65	27	35
		2	73	13	27
		3	70	5	30

TABLE V

ANALYSIS OF VARIANCE OF EMERGENCE DATA GIVEN IN TABLE IV

Source of variance	D.f.	Mean square
Replicates	3	5.33*
Disease	1	420.00**
Packing	1	16.00**
Disease × packing	1	25.00**
Error	41	1.98

*Significance, * = 5% point; ** = 1% point.*

The analysis indicates that packing of the soil caused a significant reduction in emergence of diseased seed. When the soil was packed, the amount of seedling blight and stunting increased. Hynes (10) found that the *H. sativum* and *F. culmorum* type of root rot of wheat in a district of New South Wales in 1932 was extensive on fallow that had a limited cultivation previous to

sowing, while in those cases where the fallow had been cultivated four to six times during the eight or nine months prior to seeding, the disease was slight. He attributed the partial control in the latter case to a combination of biological control and increased crop vigour. Hynes (10) and Christensen (2) considered that antibiosis plays a small role in limiting the amount of injury from *H. sativum*, especially where the fungus is seed-borne. The results of Experiment IV support this view. It seems, therefore, that the increase in injury by *H. sativum* when the soil is packed cannot be ascribed entirely to suppression of biological activity. The writer considers that packing exerted an adverse mechanical effect upon weak seedlings, the influence of which was greater than its physiological effect upon the fungus or factors that affect the fungus. A characteristic feature of seedling injury by this fungus is weakening and distortion which limits the ability of the seedlings to emerge. Any factor that increases the resistance of the soil tends to reduce the emergence. As shown in Table IV, packing of the soil under greenhouse conditions caused reduction in emergence when diseased seed was sown.

D. Fertilization of the Soil

Dosdall (3) found from field experiments, designed to show the effect of fertilizers on infection in wheat and barley by *H. sativum* that there was no correlation between the amount of disease and any particular fertilizer. Russell and Sallans (18) in studying the incidence of common root rot on wheat fertilized with phosphate fertilizers, found that fertilized wheat usually exhibited a somewhat higher disease rate than unfertilized wheat. There was a tendency for this to occur only where there was an increase in yield also. Greaney (7) concluded that deficiencies of phosphorus failed to influence the severity of infection of wheat seedlings by *Fusarium culmorum* (W. G. Smith) Sacc., while an excess of phosphorus appeared to accentuate it. Broadfoot and Tyner (1) concluded that extremely small concentrations of phosphorus had no effect on root rot of wheat caused by *H. sativum*, and excess phosphorus produced no significant reduction in the amount of disease. Vanterpool (22, pp. 234–241) found that addition of phosphate fertilizers to "browning" soil increased the dry weight of wheat plants and the number and length of crown roots. The ratio of healthy to diseased roots was increased also.

Experiment V

The effect of adding ammoniated superphosphate (2-19-0) fertilizer to the soil when barley infected with *H. sativum* was sown, was studied in the greenhouse. A portion of the seed was treated with Ceresan to represent healthy seed. The seed was sown in loam soil in six-inch pots and 0.5 gm. of the fertilizer was mixed with the soil surrounding the seeds. The pots were set up in a Latin square formation including four replicates and four treatments. Data on emergence, disease rating, and dry weight are presented in Table VI and the analyses of variance are given in Table VII.

TABLE VI

EFFECT OF FERTILIZATION OF SOIL ON EMERGENCE, DISEASE, AND DRY WEIGHT OF BARLEY SEEDLINGS FROM HEALTHY SEED AND SEED INFECTED WITH *H. sativum*

Seed	Soil	Test	Emergence, %	Disease rating, %	Dry weight per plant, mg.
Healthy	Unfertilized	1	99	0	20.0
		2	98	0	20.4
Healthy	Fertilized	1	98	0	23.1
		2	97	0	21.0
Diseased	Unfertilized	1	80	12.3	15.8
		2	77	11.2	15.1
Diseased	Fertilized	1	73	16.0	16.5
		2	70	12.0	18.4

TABLE VII

ANALYSES OF VARIANCE OF DATA SUMMARIZED IN TABLE VI

Source of variance	D.f.	Mean squares	
		Emergence	Dry weight
Replicates	3	6.25	98.54
Disease	1	264.50*	12720.13*
Fertilization	1	8.00	1682.00**
Disease × fertilization	1	4.50	544.50
Error	25	2.79	302.84

Significance: * = *5% point;* ** = *1% point.*

Emergence of diseased seed in the fertilized pots was lower than in the unfertilized pots but the difference was not significant. It was caused by increased pre-emergence blighting. The increase in dry weight of the seedlings by fertilization was significant. The results show that infection of barley seedlings by *H. sativum* may be more severe in the presence of a phosphate fertilizer than without it, but that the surviving seedlings grow more vigorously than unfertilized seedlings.

E. Soil Atmosphere

Russell and Appleyard (17) found that the soil air commonly contains less oxygen and more carbon dioxide, usually also more nitrogen than atmospheric air but the differences are often small. However, the soil air is not constant in composition but changes somewhat from day to day and to a greater extent from season to season; the latter may be so great as to mask altogether the local fluctuations. Whatever the history of the soil, its atmosphere in spring, and to a less extent in autumn was characterized by high

amounts of carbon dioxide while in summer and winter the amounts were much lower. The temperature and moistness of the soil affect the composition of the soil atmosphere as do manuring and cropping. The results of analysis of four arable Rothamstead soils gave the following percentages by volume in the soil atmosphere: carbon dioxide, 0.25%; oxygen, 20.6%; nitrogen, 79.12%. Atmospheric air was found to contain 0.03% carbon dioxide, 20.9% oxygen, and 79% nitrogen. Thus the chief difference was the amount of carbon dioxide in air and in soil. It reached 2% in manured soils during the biologically active season. Carbon dioxide content of garden soils may sometimes be so high as to be detrimental to the root development of some common species (15). Few plants can survive long in soil or water low in oxygen. An important source of oxygen is rain water which is highly charged with the gas (23). Hall, Brenchley, and Underwood (8) found that the rate of root growth and top growth in both soil and water culture could be increased by pulling a stream of air through the medium. In an experiment, they found that the average dry weight of barley plants in non-aerated water cultures was 1.31 gm. and in aerated cultures, 2.12 gm. According to Miller (14) depletion of the oxygen supply of the soil interferes with the process of respiration in the roots, through injury to the protoplasm, causing it to fail as an absorbing organ. Stiles and Jörgensen (20) found that aeration of a nutrient solution increased the rate of growth of barley. Garrett (5) found that by aerating acid soils, they could be made as favourable as alkaline soils for the growth of *Ophiobolus graminis* Sacc. along the roots of wheat seedlings. This finding was in agreement with his theory that the growth of the fungus in acid soils is retarded by the accumulation of respiratory carbon dioxide. Fellows (4) studied the effect of a scarcity of oxygen and an excess of carbon dioxide in the atmosphere surrounding pure cultures of *O. graminis*. In a liquid medium, growth diminished gradually as the oxygen concentration decreased; on a solid medium marked diminution did not occur until oxygen was below 6%. A very small percentage of oxygen greatly reduced growth. The fungus grew well on both media when the carbon dioxide content was varied, although at the highest carbon dioxide concentration (18.02%), some diminution in growth occurred. Fellows believed that the variations in carbon dioxide and oxygen as found in arable soils are not great enough to affect materially the growth of *O. graminis*. Owen, Small, and Williams (16) found that an increase of the carbon dioxide concentration of the atmosphere in the greenhouse did not affect germination of tomatoes, but did increase the yield. The plants showed an increased susceptibility to *Colletotrichum atramentarium* (B. and Br.) Taub., in an atmosphere rich in carbon dioxide.

Some experiments were conducted in the laboratory to test the effect of lowering the supply of oxygen and of increasing the supply of carbon dioxide in soil or nutrient solution on the growth of barley seedlings and on the severity of infection of these seedlings by the seed-borne fungus, *H. sativum*. In addition, the effect of an atmosphere, low in oxygen, on the growth of *H. sativum* in culture was studied.

Experiment VI

In this experiment the barley was grown on perforated cork disks which floated on a nutrient solution (19) which was renewed constantly. One set of containers was aerated with a stream of air, the other set with a mixture of nitrogen and oxygen in the proportion 9:1 by volume. In each case the solution was stirred constantly. The containers were set in a water-bath which was held at constant temperature.

TABLE VIII

THE EFFECT OF REDUCING THE OXYGEN SUPPLY IN A NUTRIENT SOLUTION, UPON GROWTH AND DISEASE INCIDENCE IN BARLEY FROM HEALTHY SEED AND SEED INFECTED WITH *H. sativum*

	Seed	Aerated with:		Necessary difference*
		Air	$N_2 + O_2(9:1)$	
Germination (9 seeds)	Healthy	9	8	
	Diseased	7	7	
Height (cm.)	Healthy	9.3	6.5	3.6
	Diseased	8.5	2.0	5.1
Total root length per plant (cm.)	Healthy	20.0	17.6	17.8
	Diseased	19.5	7.2	11.3
No. seedlings infected	Healthy	0	0	
	Diseased	7	0	

* *Necessary difference, 5% level of significance between treatments.*

In this experiment, general growth of seedlings from both healthy and diseased seed was depressed by a scarcity of oxygen, but the effect was greatest in the case of diseased seed. Similarly, infection of the seedlings by the seed-borne fungus (*H. sativum*) was suppressed. This was determined by direct examination of coleoptile tissue for evidence of penetration and for lesions.

Experiment VII

In this experiment natural soil was used instead of a nutrient solution. Diseased kernels of barley were sown in moist, sifted, unpacked soil in glass tubes 12 in. long and 1 in. in diameter. These tubes were connected in series and one set was aerated with air, the other with a 9:1 mixture by volume, of nitrogen and oxygen. The air and nitrogen–oxygen mixture were first passed through Chamberland filters, then through sterile water. The tubes containing the soil and seeds were immersed in a water-bath, the temperature of which was 21° C. Data on germination, growth, and disease symptoms are summarized in Table IX.

These results are similar to those given in Table VIII. They show that a scarcity of oxygen depressed the general growth of the barley plants and decreased the amount of infection by the seed-borne fungus.

TABLE IX

THE EFFECT OF REDUCING THE OXYGEN SUPPLY IN SOIL, UPON GROWTH AND DISEASE INCIDENCE IN BARLEY FROM SEED INFECTED WITH *H. sativum*

	Aerated with:		Necessary difference*
	Air	$N_2 + O_2$(9 : 1)	
Germination (15 seeds)	15	15	
Mean height (cm.)	9.2	3.3	3.7
Total root length per plant (cm.)	26.1	18.6	20.5
No. seedlings infected:			
Shoot	11	6	
Root	13	1	

* *Necessary difference, 5% level of significance.*

Experiment VIII

The same apparatus, with tubes of natural soil, was used to test the effect of increasing the amount of carbon dioxide in the soil atmosphere on seedling growth and seed-borne infection. One lot of tubes was aerated with air, the other with a mixture of 99% air and 1% carbon dioxide, by volume. Healthy and diseased lots of seed were used, and the temperature was 21° C. The results are given in Table X.

TABLE X

THE EFFECT OF INCREASING THE CARBON DIOXIDE CONTENT OF SOIL, UPON GROWTH AND DISEASE INCIDENCE IN BARLEY FROM HEALTHY SEED AND SEED INFECTED WITH *H. sativum*

	Seed	Aeration with:		Necessary difference*
		Air	Air + 1% CO_2	
Germination	Healthy (10 seeds)	10	10	
	Diseased (30 seeds)	28	29	
Height (cm.)	Healthy	11.5	11.6	7.6
	Diseased	11.5	9.7	10.8
Total root length per plant	Healthy	49.3	48.6	11.3
	Diseased	37.4	30.2	27.8
No. seedlings infected	Healthy	0	0	
	Diseased	13	19	

* *Necessary difference, 5% level of significance between treatments.*

The amount of carbon dioxide in the soil atmosphere in the air plus carbon dioxide series was considerably greater than that which is present in air or in ordinary soil atmosphere. The results show a slight reduction in height and in root development and a moderate increase in the number of plants infected.

Experiment IX

This experiment was conducted in the laboratory. In it, *H. sativum*, originating from disks of agar bearing the mycelium, and from barley kernels inoculated with the fungus during maturation was exposed to two kinds of atmosphere, at constant temperature. Two sets of agar cultures (2% potato dextrose agar) were placed in sealed containers through which air and a 9:1 mixture of nitrogen and oxygen, respectively, were passed. Both gases were filtered through cotton and a Chamberland filter and humidified by passage through sterile water. The covers of the Petri dishes were removed before they were placed in the chambers, in order to expose the fungus directly to the gas. The chambers were set in a water-bath, the temperature of which was maintained at 17° C. On the fifth day, the diameter of the colonies was measured and general observations on the thickness of the aerial mycelium and the progress of sporulation were made. These data are given in Table XI.

TABLE XI

THE EFFECT OF REDUCING THE OXYGEN CONTENT OF THE ATMOSPHERE UPON THE GROWTH OF *H. sativum* ON POTATO DEXTROSE AGAR

Inoculum	Diameter of colonies, cm.		Necessary difference*
	In air	In $N_2 + O_2$(9 : 1)	
Mycelium	3.12	3.00	0.62
Diseased seeds	2.11	1.95	0.14
Sporulation	Well advanced	Trace	

* *Necessary difference, 5% level of significance between treatments.*

There was little difference in the radial growth under the two sets of conditions, but the mycelial mat was noticeably thicker in air than in the nitrogen–oxygen mixture, and it was sporulating more freely.

The results of the experiments that deal with the effect of changes in atmosphere, because of their limited scope, are considered to be indicative rather than conclusive and to agree in some respects with those of other investigators. They indicate that changes in the amount of oxygen and carbon dioxide in the atmosphere in which barley kernels are germinating may affect the growth of the seedlings themselves and the development and parasitism of a seed-borne fungus. Generally reduction in oxygen caused stunting of the seedlings and reduced the activity of the fungus and this is interpreted to be the result of slower metabolism of both organisms. There was some reduction in seedling growth and a slight increase in the activity of the fungus when the amount of carbon dioxide in the atmosphere was increased to 1.25%. This appears to be similar to a condition in soil that is only moderately well aerated. The top and root growth of plants from diseased kernels was reduced more by lack of oxygen than that of the plants

from healthy kernels. The interpretation placed on this is that the kernels were weaker to begin with, because their embryos competed with a fungus for food supplies during maturation. In addition, there is the possibility that this fungus excreted an injurious toxin which might pass the semi-permeable layer of the kernel. Vanterpool (21) found that *H. sativum* on certain media, produces a substance toxic to wheat.

Discussion

When this disease was studied under controlled conditions, it was found that certain environmental factors affected its intensity and that these factors affected both host and parasite. Temperature and moisture were closely associated. For instance, high temperature (32° C.) in itself was not limiting, but when associated with a lack of moisture it reduced the amount of infection. Seedling growth was poor at that temperature also. The fungus caused severe blighting at temperatures from 10° to 30° C. as long as there was sufficient moisture for good seedling growth. The strongest plants and best stand were obtained from diseased seed when the temperature was between 15° and 18° C. This temperature is closer to the optimum for barley than it is to the optimum for *H. sativum*. It has been concluded that seeding of diseased barley might better be done when conditions favoured the plant, i.e., when temperature was about optimum and moisture conditions good.

Well tilled and well aerated soil were more favourable to the diseased barley than packed or water-logged soil, or soil poor in oxygen or containing an excess of carbon dioxide. This was interpreted to mean that the favourable growing conditions were primarily beneficial to the barley seedlings rather than exerting an antibiotic effect on the fungus through intense biological activity. Enrichment of the soil with a phosphate fertilizer increased the disease but increased the vigour of the seedlings also, the net result being a slight reduction in stand and a slight increase in dry weight.

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NON-STERILE SOIL AS A MEDIUM FOR TESTS OF SEED GERMINATION AND SEED-BORNE DISEASE IN CEREALS[1]

By J. E. Machacek[2] and H. A. H. Wallace[3]

Abstract

Experiments with non-sterile soil in large seed beds showed that such soil, if maintained in proper condition, could be used with satisfactory results for tests of seed germinability and of certain kinds of seed-borne diseases in cereals.

Non-sterile soil was more easily handled than autoclaved soil and it did not require fresh preparation for each planting.

The amount of soil-borne infection in the seedlings was neligible when friable, non-sterile soil was kept moist and at 20° C.

A comparison of the results from 120 different lots of seed showed that a test of seed in non-sterile soil was equal in some respects, and superior in others, to a test on moist paper or to a test on nutrient agar in Petri dishes as a means of measuring seed germinability, seed-borne disease, and physical injury to the seed. With barley seed, a plating test, in addition to the soil test, was found necessary to indicate the amount of seed-borne infection due to *Helminthosporium teres*.

A table of tentative recommendations, based on tests with several thousand seed lots, is given. Seed disinfection is recommended where the smut spore load exceeds 1 : 128,000 or where seed decay or seedling blight reduces the percentage of healthy seedlings from non-disinfected seed below 91%. Increases in rates of seeding are recommended when the percentage of healthy seedlings, even after seed disinfection, is less than 91% but more than 50%. Seed germinating 50% or less after disinfection is to be discarded.

Introduction

The testing of seed to measure the amount of seed-borne disease is a comparatively new and unstandardized procedure. The methods currently used include (1) the visual examination of the seed to determine its condition, (2) the examination of seed washings to determine the load of fungus spores carried on the seed, (3) the plating of the seed on nutrient agar or moist filter paper to determine the kind of fungi present within the seed, and (4) the planting of seed on some moist substrate, such as paper, peat moss, sterile sand, or sterile soil, to determine the percentage of diseased seedlings. Tests for the presence of seed-borne disease are often made in conjunction with tests of seed germinability.

While the above methods of testing seed in the laboratory for seed-borne disease possess individual merits, the common fault of most is that the seed is usually tested under conditions quite different from those under which it ordinarily germinates in the field. Often unfavourable conditions for growth, such as poor light, crowding, etc., weaken the seedlings to such a degree that they become subject to attack by relatively innocuous micro-organisms.

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Besides, owing to the absence of competition from saprophytes in some tests, the pathogenicity of weak pathogens may become exaggerated. In plating tests, too, it is usually impossible to separate the pathogenic from the non-pathogenic strains of the same fungal species. To overcome these difficulties, the writers considered using other methods of testing seed for the presence of seed-borne disease, and came to the conclusion that the use of non-sterile soil as a seed-testing medium offered considerable promise.

A review of the available literature yielded very little information regarding the use of non-sterile soil for seed-testing purposes. Flats or beds of non-sterile soil have been frequently used by investigators in connection with tests of seed disinfectants, but relatively seldom for tests of seed-borne disease. The general impression gained from the literature was that non-sterile soil harboured phytopathogens in dangerous quantities, and thus rendered such soil unfit for seed tests. Crosier and Patrick (3), for instance, opposed germination tests in soil, their data suggesting that the results from such soil were an index of the soil condition rather than of seed vitality, particularly if the soil did not have a low "inoculum potential."

The evidence from field experiments, however, suggests that non-sterile soil is not always a breeding ground for plant diseases. With cereals, conditions adverse to plant growth, such as high soil salinity (7), low soil moisture (6, 13), soil acidity (13), lack of nutrients (13), appear to intensify the attacks by root rotting, soil-borne fungi. In addition, recent unpublished observations by the writers showed that deep seeding also increased the severity of root rot. On the other hand, disease-free seed, when sown thinly and not too deeply in good soil, produced vigorous plants seldom damaged by soil-borne, root rotting fungi. However, under the favourable conditions just mentioned, seed infected with either *Helminthosporium sativum* P. K. & B. or *Fusarium culmorum* (W. G. Sm.) Sacc. either failed to germinate or produced weak plants that were lesioned at the base.

In view of the fact that under certain favourable field conditions cereal plants remained free from soil-borne infection, it seemed to the writers that there was much to be learned about the behaviour of non-sterile soil under greenhouse conditions. On this account, most of the experimental work described here, and carried on during the years 1939, 1940, and 1941, was devoted to a study of the conditions that would permit an attack of cereal seedlings by seed-borne fungi but would not permit an attack by soil-borne fungi. To conserve space, many of the experimental details are omitted. A part of the investigation was devoted to a comparison of the results from seed tests in non-sterile soil with those obtained by other methods.

Development of the Non-Sterile Soil Test

Preparation of the Seed Beds

Wooden frames, made of 2-in. by 6-in. planks, were set upon greenhouse benches. Only the centrally located benches were utilized because it was thought that the lateral benches, heated below by steam pipes, would make

difficult the controlling of soil temperature. Convenient widths for the seed beds were found to be 30 or 60 in., the narrower ones for small-seeded crops, such as flax, and the wider ones for cereals. The frames were filled with artificially prepared, friable soil.

A simple test of friability for unknown soils was devised. In the test, the unknown soil, air-dry and sifted, was mixed with fine sand in various proportions and the mixtures were put separately into 4½-in. ordinary earthenware pots. The filled pots were set in pans of water and left there until moisture had reached the surface of the soil. They were then removed from the pans and placed upon a greenhouse bench to drain for about an hour. When the excess water had drained out, the pots were inverted upon a flat surface and lifted from the conic frusta of moist soil within. Two or three days later, when these frusta of soil had become dry, a light, flat-bottomed dish was placed upon the upper plane of each and sand was poured into the dish until the mould of soil beneath collapsed from the weight. A mixture of soil and sand collapsing under a weight of 3 to 4 lb. was found to be friable to the desired degree. With sandy soils, lacking in organic matter, the soil was usually too porous at this degree of friability, and, with peaty soils, the soil was too retentive of moisture. Such extreme soils were not used when large scale tests with seeds were made.

After the proper mix was determined for a soil, enough of the mixture was prepared to fill the required number of seed beds. In filling the frames, a basal, 4-in. layer was put on the bottom. This layer was levelled and soaked with water, and covered with a layer of prepared soil ½ in. thick. This second layer was then levelled and furrowed transversely across the bed by pressing a bevel-edged board into it at 2-in. intervals to the depth of ½ in. Seed was planted in these furrows, covered with another ½ in. layer of prepared soil, and the finished seed bed was given a second, lighter watering.

The method of watering the seed beds was found to have a marked effect upon the uniformity of stand in the seedlings. When the thorough watering of the basal layer was omitted and the seed bed was watered only after the seed was planted and covered, the stand of seedlings was usually uneven and "spotty." On the other hand, with the watering of the basal layer, the stand was always very uniform. Also, different crops were found to require different methods of watering. With cereals, the method just described proved to be satisfactory, but with dicotyledonous crops, such as flax and certain small-seeded vegetables, the seedlings experienced difficulty in penetrating the crust of soil formed after a surface watering. To prevent a crust from forming until after the seedlings had emerged and to conserve moisture, the seed beds were covered with sheets of paper after they were sown. Following the emergence of the seedlings, the paper was removed and the soil then watered as needed.

Preparation of the Seed for Planting

Healthy seed, as well as seed damaged by fungal infection, mechanical injury, frost, and sprouting was used in the experiments described in this paper. This seed, collected from farmers in various parts of Canada, was subjected to a preliminary cleaning and then stored in tin boxes. When required in an experiment, 100-kernel lots of seed—the number of lots depending on the experiment—were counted out from the cleaned seed and planted in a seed bed, one 100-kernel lot to a row.

In experiments where disinfected as well as non-disinfected seed was used, two 100-kernel lots were counted out from each seed lot and placed separately in small coin envelopes. One of these 100-kernel lots was mixed with a dust disinfectant, applied to the seed in excess to ensure complete coverage of the seed, the surplus dust being removed by shaking the mixture in a small, wire strainer. For example, a dust disinfectant, useful for experimental purposes, was made by mixing one volume of Ceresan (5% ethyl mercury phosphate) with two volumes of powdered talc. At this dilution Ceresan did not reduce the growth of seedlings, but it prevented their infection by seed-borne or soil-borne root rotting fungi. Lower dilutions tended to stunt the seedlings, while higher dilutions gave less control of root rotting fungi. Other mercurial dusts in suitable dilutions also gave satisfactory results.

Factors Influencing the Results from Seed Beds of Non-sterile Soil

During the course of preliminary experimentation, it was found that at least three different factors, (1) soil moisture, (2) soil temperature, and (3) the time interval between the planting of seed and the collection of data, directly or indirectly influenced the results obtained from beds of non-sterile soil. As already mentioned, the method of watering influenced the uniformity of stand in the seed beds. It also affected the amount of basal lesioning caused by soil-borne pathogens in the seedlings. When the soil was kept too moist, the seedlings were frequently attacked by species of *Pythium*, and "damped-off." This form of damage occurred most often in peaty soil. On the other hand, when the soil was kept too dry, the seedlings were occasionally attacked by *Rhizoctonia Solani* Kühn. This latter observation is in accord with the findings of Hynes (6) concerning wheat and oats, and with those of Sanford (12) concerning potatoes, but it is opposed to the findings of Abdel-Salam (1), Alexander *et al.* (2), and Gratz (4) concerning vegetables.

To ascertain the most suitable conditions of soil moisture, a large seed bed of non-sterile soil was divided into three sections, the basal layer in each was watered copiously and then the sections were sown with disease-free seed of wheat, oats, and barley. After seeding, each section was watered in a different way. One of the sections received no further watering, the second section received a light watering immediately after seeding, and the third section was watered whenever the surface of the soil appeared to be dry. At the end of 10 days, when the plants were dug up for examination, the soil in the first section was quite dry, in the second section it was dry only at the surface,

while in the third section it was moist throughout. The results from four replicates of the test, with four relatively disease-free seed lots each of wheat, oats, and barley are summarized in Table I. These results show that, with a decrease in the amount of water given a seed bed, there was an increase in the percentage of blighted seedlings in wheat and barley, while there appeared to be no effect on oats. The amount of disease, however, was not very great, even in the driest seed bed. The results suggested that soil-borne disease developed least in moderately moist soil, and therefore the seed beds in later experiments were kept in this condition continually.

TABLE I

EFFECT OF THREE METHODS OF WATERING UPON THE PERCENTAGE OF SEEDLINGS WITH BASAL LESIONS FROM DISINFECTED AND NON-DISINFECTED SEED OF WHEAT, OATS, AND BARLEY

Method of watering	Wheat		Oats		Barley		Mean
	Disinfected	Non-disinfected	Disinfected	Non-disinfected	Disinfected	Non-disinfected	
Soil kept moist	0.36	4.46	0.12	2.44	0.76	1.45	1.59
Soil watered once	0.35	5.63	0.25	1.71	0.63	3.45	2.00
Soil not watered after seed planting	2.73	5.23	0.25	2.87	4.31	5.56	3.49
Necessary difference (5% level)	2.13	2.13	2.13	2.13	2.13	2.13	0.74

Soil temperature had only a small and indirect effect on the development of soil-borne disease. When disease-free, as well as diseased (40% of kernels infected by *Helminthosporium sativum*), undisinfected seed of Pentad wheat was planted in non-sterile, and in autoclaved, prepared soil, there was an increase in seedling blight with a rise in temperature. In view of the results obtained from the experiment concerning soil moisture, it appeared that the marked loss of soil moisture, associated with high soil temperature, was the actual cause of the increase in disease. When this experiment was repeated and the soil was kept moist, it was found that soil temperature within the range 10 to 30° C. had apparently no effect on the amount of disease, for an analysis of the experimental data (summarized in Table II) showed the differences between the percentages of blighted seedlings at the different soil temperatures to be statistically insignificant. The temperature of the soil, however, had a marked effect on seedling growth, the rate of growth increasing with the temperature. A temperature of 20° C. was found to be the most suitable for general use, for, at that temperature, the growth of seedlings was rapid, and the rate of evaporation of moisture from the soil was relatively slow.

TABLE II

EFFECT OF SOIL TEMPERATURE ON THE PREVALENCE OF BLIGHT IN THE SEEDLINGS FROM DISEASE-FREE AND FROM DISEASED PENTAD WHEAT PLANTED IN NON-STERILE AND IN AUTOCLAVED SOIL (MEANS OF FOUR REPLICATES)

Soil temperature	Percentage of blighted seedlings			
	Disease-free seed		Diseased seed	
	Non-sterile soil	Autoclaved soil	Non-sterile soil	Autoclaved soil
15° C.	0.3	0.4	30.5	55.2
20° C.	2.4	0.0	14.8	38.6
25° C.	4.4	2.8	19.6	45.7
30° C.	2.2	7.2	13.3	41.0
Necessary difference (5% level)	15.2	15.2	15.2	15.2

When seed beds of non-sterile soil were kept moist and held at approximately 20° C., the percentage of seedlings with basal lesions increased with the lengthening of the interval between the time when the seed was planted and when the seedlings were dug up. Usually, up to the sixth day, the amount of disease was small, but from the sixth day to the 12th day the increase in disease was quite rapid. On the 12th day, the percentage of seedlings with basal lesions equalled, or slightly exceeded, the percentage of wheat or barley kernels found infected, by the plating-out method, with *Helminthosporium sativum*, or, in the case of oats, with *H. Avenae* Eidam. From the 12th to the 20th day, there was only a slight increase in basal lesioning, but after the 20th day, a second rapid increase in disease occurred. The results of an experiment showing this trend in the development of basal lesioning are summarized in Table III.

TABLE III

AGE OF PLANT IN RELATION TO THE PERCENTAGE OF SEEDLINGS WITH BASAL LESIONS IN THREE LOTS OF PEATLAND BARLEY, INFECTED WITH DIFFERENT AMOUNTS OF *Helminthosporium sativum*

Age of plants (days)	Percentage of seedlings with basal lesions		
	Uninfected seed	Seeds infected, 16%	Seeds infected, 47%
8	1.2	10.5	47.7
10	2.0	8.5	56.5
12	6.0	9.5	48.6
14	3.0	15.5	60.0
16	4.7	15.2	57.0
18	6.5	16.5	59.5
20	8.5	14.7	69.2
22	7.7	25.2	75.3
Necessary difference (5% level)	5.73	5.73	5.73

The Effect of Soil Amendment or Soil Renewal

When sterile soil is used for seed beds, it is usually necessary to refill the seed beds with sterile soil for each planting. With non-sterile soil, the original soil may be left in the seed beds after the plants are dug up or it may be replaced with fresh non-sterile soil. Leaving the soil in the seed beds involves the least expenditure of labour, but since the repeated use of the same soil may result in a deterioration in its quality, an occasional changing of the soil may be necessary. The experiment described below indicates that, with tests of cereal seed, such changes need not be frequent.

A large bed of non-sterile soil, the soil of which had been used continuously for a period of six months, was divided by means of boards into four equal sections. The soil in one section was replaced with "new" soil of the same kind. To the soil in the second section, ammonium phosphate fertilizer (11-48) was added at the rate of 11 gm. to two Imperial gallons of soil. The soil in the third section was watered with a solution of thiamin hydrochloride (vitamin B_1) in the concentration recommended by the manufacturer (15 International Units in 1 gal. of water) instead of with water alone. The soil in the fourth section was left unchanged and unamended. In each section, two lots each of wheat, oats, and barley were sown, and the seed beds were kept moist and at about 20° C. Ten days after seeding, the seedlings were lifted, counted, examined for the presence of basal lesions, and weighed while green. The results of this experiment showing the averages for each section of the bed (four successive plantings in a section) are shown in Table IV. These results indicate that the addition of soil amendment or changing the soil influenced neither the percentage of seeds germinating nor the green weight. The failure to affect the green weight showed that the soil had not become seriously depleted of nutrients even after six months of continuous use. There was, however, a significant difference between the mean percentages of plants with basal lesions, that is, more seedlings were infected in the "new" soil than in the "old" soil.

TABLE IV

THE INFLUENCE OF SOIL AMENDMENT AND SOIL RENEWAL ON THE PERCENTAGE OF SEEDS GERMINATING, THE PERCENTAGE OF SEEDLINGS WITH BASAL LESIONS, AND THE TOTAL GREEN WEIGHT OF SEEDLINGS FROM 100 SEEDS

Factor studied	Unamended "old" soil	Fertilizer added	Vitamin B_1 added	"New" soil	Necessary difference (5% level)
Germination	94.2	94.6	94.5	93.6	1.06
Seedlings with basal lesions	0.4	0.8	0.7	1.9	0.55
Green weight (gm.)	22.7	22.1	22.5	21.7	1.21

Amount of Variability Between Different Seed Beds and Plantings

Beds of non-sterile soil, prepared, planted, and maintained in the way already described, showed very little variability in the results obtained from them. For instance, when a seed lot of disease-free wheat was planted in each of seven different seed beds located in four different greenhouse sections, and replicate plantings were made one, two, and three months after the initial planting, the variability in germination and disease was small, and the differences were statistically insignificant. In each instance, germination was over 90%, the range between the maximum and minimum percentages being seven per cent for disinfected seed and nine per cent for non-disinfected seed. The range in the percentage of seedlings with basal lesions was even less, being only one per cent in the plants from disinfected seed and four per cent in the plants from non-disinfected seed. In another trial, with 12 lots of wheat seed differing in amount of disease, the differences between seed beds and plantings were also statistically insignificant. It was assumed, therefore, that if the seed beds were maintained in the proper condition, the results of the non-sterile soil test were unlikely to be affected by minor differences between greenhouses.

The Collection of Data from Plantings in Beds of Non-sterile Soil

After trials with several hundred different lots of seed of wheat, oats, and barley that had been planted in beds of non-sterile soil, it was concluded that by following a relatively simple procedure a great deal of information could be obtained for any seed lot. Firstly, by examining the rows of seedlings growing in the seed beds, it was often possible to identify the varieties of wheat in lots of mixed seed, or the variety when the varietal name of the seed was not given. For instance, the varieties Thatcher, Renown, and Regent, which embrace the bulk of the wheat crop in Manitoba, were readily distinguishable. In the seedling stage, Thatcher was found to have smooth leaves and practically no reddish pigment in the coleoptile. Renown, on the other hand, was found to possess both pigment and rough leaves. Regent differed from Renown in that the pigment was generally absent, but the leaves were rough. Among the durum wheats, none of the varieties tested had rough leaves and only the variety Golden Ball showed any pigmentation. In oats and barley, characters definitely distinguishing one variety from another were not found. Secondly, the germinability of seed could be determined by counting the seedlings that had emerged from the soil. Ordinarily, because the amount of basal lesioning was used as an index of seed infection by root rotting fungi, this count of seedlings was delayed until after they were dug from the soil and cleaned. As each lot of seedlings was dug, they were divided into two groups, one containing seedlings with basal lesions and one containing seedlings that showed no lesions. Each group was then separated into three subgroups, the first containing vigorous plants, the second containing weak plants, and the third containing distorted plants. Weak plants were generally found to arise from shrunken, diseased, or broken seed, while the distorted seedlings originated from seed damaged by frost, sprouting, or, as in oats,

from seed infected with *Helminthosporium Avenae*. The percentage of healthy, vigorous seedlings was taken as an index of the seed value of any particular seed lot. Thirdly, when a row of disinfected seed as well as a row of non-disinfected seed of the same seed lot was planted, it was possible, by comparing the results from the two rows, to assess the value of seed disinfection for the particular seed lot. The test with disinfected seed was taken to show the maximum germinability of the seed. Generally, unless the seed was free from infection by root rotting fungi, seed disinfection increased the percentage of vigorous plants.

Comparison of the Results Obtained by the Non-Sterile Soil Method of Testing Seed with Those Obtained by Other Methods

When the non-sterile soil method of seed testing had been developed to a point where repeated tests with a seed lot gave relatively uniform results, this method was compared with other methods of seed testing. This comparison was made with the object of determining the relative accuracy of the different methods, as well as of determining the relative amounts of time, labour, and costs of materials consumed in following them.

The first comparison was between non-sterile soil and autoclaved soil. In Table II the data show that, with diseased wheat, fewer seedlings became diseased in non-sterile soil than in soil that had been autoclaved. As this result was obtained from soil in relatively small (1 gal.) containers, the result was not thought wholly comparable to the result obtainable from large seed beds, and therefore the experiment was repeated, but on a larger scale. To obtain comparable conditions, a large seed bed was partitioned into three sections, one of which held non-sterile prepared soil, another held prepared soil that had been autoclaved at 15 lb. pressure for three hours, and a third held a 4-in. layer of non-sterile soil on the bottom and a 1-in. layer of autoclaved soil, in which the seed was planted, at the top. In each section of soil, disinfected and non-disinfected seed from two lots each of relatively disease-free seed of wheat, oats, and barley were planted. Ten days later, the seedlings were dug, counted, and examined for the presence of basal lesions. The results (averages of four replicates) are summarized in Table V. They show that, contrary to the general belief, less disease developed in non-sterile soil than in sterile soil, while the germination of seed was equally good in both. In the two-layer soil, the amount of disease was slightly more than in the other two soils. A slightly different result was obtained from a similar experiment, but with diseased seed. In this second experiment, there was (Table VI) least disease in the two-layer soil, while the results from non-sterile soil and from sterile soil were very much alike. The results of these two experiments show, therefore, that a test of seed in non-sterile soil may be expected to give results very similar to a test of seed in sterile soil. This being the case, a test in non-sterile soil is to be preferred, as the expenditure of time and labour involved in soil sterilization is avoided.

TABLE V

EFFECT OF DIFFERENT SOIL TREATMENTS ON THE PERCENTAGE OF SEEDS GERMINATING AND THE PERCENTAGE OF SEEDLINGS WITH BASAL LESIONS IN RELATIVELY DISEASE-FREE SEED OF WHEAT, OATS, AND BARLEY

Factor studied	Seed treatment	Crop	Soil treatment		
			Non-sterile	Autoclaved	Non-sterile and autoclaved
Germination	Disinfected	Wheat	96.5	96.0	95.5
		Oats	97.5	98.0	97.5
		Barley	98.0	97.5	97.5
	Non-disinfected	Wheat	96.0	97.5	95.5
		Oats	98.0	98.0	95.5
		Barley	96.5	97.0	97.0
	Mean*		97.0	97.3	96.4
Seedlings with basal lesions	Disinfected	Wheat	0.0	0.1	0.2
		Oats	0.0	0.0	0.1
		Barley	0.0	0.0	0.3
	Non-disinfected	Wheat	1.0	1.8	1.7
		Oats	1.0	1.3	1.9
		Barley	0.8	2.1	2.7
	Mean**		0.46	0.88	1.15

* *Difference between means not significant.*
** *Difference of 0.57, significant at 5% level.*

As already mentioned, another common method of testing seed for seed-borne disease is to plate the seed out on nutrient agar. This method, while showing the degree to which the seed is infected with pathogenic as well as non-pathogenic fungi, does not indicate better than roughly seed germinability.

The data in Table VI indicate that the percentage of seeds found, by a plating test described elsewhere (8), to be infected with seedling blight fungi (*Helminthosporium sativum* and *H. Avenae*) is closely related to the percentage of seedlings with basal lesions when the diseased seed was planted in non-sterile soil. These data were obtained from tests with only a few seed lots and were not considered to be conclusive. When, however, the results of plating tests with several hundred seed lots of wheat, oats, and barley were compared with the results obtained from the same seed lots planted in non-sterile soil, the relation became more pronounced. As shown in Table VII, an increase in the percentage of seeds infected with either *H. sativum* or *H. Avenae* was accompanied by an increase in the percentage of seedlings with basal lesions. In wheat, there was a tendency for the percentage of infected seeds to be higher than the percentage of lesioned seedlings, a tendency that was reversed in oats. In barley, the percentages were nearly equal. The

TABLE VI

EFFECT OF DIFFERENT SOIL TREATMENTS ON THE PERCENTAGE OF SEEDS GERMINATING AND ON THE PERCENTAGE OF SEEDLINGS WITH BASAL LESIONS IN DISEASE-FREE AND DISEASED SEED OF WHEAT, OATS, AND BARLEY

Soil treatment	Percentage of diseased kernels in seed*														
	Wheat					Oats					Barley				
	0	0	2	24	Mean (*A*)	0	1	3	20	Mean (*A*)	2	6	21	34	Mean (*A*)
*Percentage of seeds germinating***															
Non-sterile	97	97	95	76	91.2	97	98	98	95	97.0	94	99	96	97	96.5
Autoclaved	98	95	97	86	94.0	97	98	98	93	96.5	98	98	98	97	97.7
Two-layer	95	97	96	88	94.0	98	95	96	94	95.7	95	99	94	98	96.5
Mean (*B*)	96	96	96	83		97	97	97	94		95	98	96	97	
*Percentage of seedlings with basal lesions****															
Non-sterile	1.0	5.0	1.0	27.8	8.7	0.2	0.5	1.5	26.5	7.2	0.0	1.0	24.2	35.0	20.0
Autoclaved	1.5	1.5	2.2	33.3	9.6	0.0	2.5	0.2	27.2	7.5	0.0	2.0	19.6	26.5	12.0
Two-layer	2.0	0.7	1.5	27.5	7.9	0.2	1.2	2.7	24.3	7.1	0.5	1.5	11.5	20.2	8.4
Mean (*B*)	1.5	2.4	1.5	25.6		0.1	1.4	1.4	26.0		0.2	1.5	18.4	27.3	

* *Wheat and barley infected with Helminthosporium sativum, oats infected with H. Avenae.*

** *Difference for means (A) not significant, but for means (B) a difference of 1.71, significant at 5% level.*

*** *A difference of 2.01 for means (A) and a difference of 2.83 for means (B), significant at 5% level.*

difference, in wheat, was thought due to an infection of some of the seed with strains of *H. sativum* incapable of causing basal lesioning in non-sterile soil under the conditions of the test. On the other hand, the difference in oats seemed due to a superficial infection by *H. Avenae* in some seed-lots. This superficial infection, proved by other tests, was thought to be destroyed when the oat seed was surface sterilized prior to being plated out. The amount of infection of the seed with other pathogenic fungi, such as *H. teres* Sacc. in barley and *Fusarium* spp. in wheat, oats, and barley, apparently had no relation to the amount of basal lesioning. When the percentage of seeds infected with either *H. sativum* or *H. Avenae* was added to the percentage of seeds infected with *H. teres* or *Fusarium* spp., or both, the closeness of the relation between seed infection and basal lesioning decreased. These results were taken to mean that, of the fungi considered, only *H. sativum* and *H. Avenae* attacked the seedlings of cereal crops in non-sterile soil. Other seed-borne, seedling blight fungi, such as *Fusarium culmorum* (W. G. Sm.) Sacc. or *Gibberella Saubinetii* (Mont.) Sacc., were not encountered in any of the seed lots examined, and for this reason a comparison between a plating test

TABLE VII

THE RELATION BETWEEN THE PERCENTAGES OF SEEDS FOUND INFECTED, BY THE PLATING TEST, WITH PATHOGENIC FUNGI, AND THE PERCENTAGES OF SEEDLINGS WITH BASAL LESIONS

Fungus	Percentage of seeds infected with fungus	Average percentage of seedlings with basal lesions		
		Wheat	Barley	Oats
Helminthosporium sativum	0– 5	1.85	2.60	13.40
	6–10	4.34	4.17	5.20
	11–20	5.28	7.70	*
	21 +	*	17.83	*
Helminthosporium teres	0– 5	**	3.52	**
	6–10	**	0.57	**
	11–20	**	1.66	**
	21 +	**	3.66	**
Helminthosporium Avenae	0– 5	**	**	7.42
	6–10	**	**	15.18
	11–20	**	**	24.60
	21 +	**	**	43.00
Fusarium spp. ***	0– 5	0.92	3.30	12.56
	6–10	2.08	4.55	12.65
	11–20	0.00	2.25	10.52
	21 +	*	*	8.00

* *No seed lots with this class of infection.*
** *Fungus not found in seed of this crop.*
*** *Fusarium Poae, F. Avenaceum, F. Scirpi.*

and a non-sterile soil test for these fungi could not be made. It is thought, on the basis of previous experience with these fungi, that the relation would be similar to that already found for *H. sativum* and *H. Avenae.*

In a third comparison, the results from non-sterile soil were compared with those obtained when non-disinfected seed was germinated on moist paper in incubators maintained at constant temperature and humidity. For purposes of comparison, a group of 40 seed lots each of wheat, oats, and barley were selected from among a large number of samples collected from the grain growing areas throughout Canada. The selected seed lots represented healthy seed as well as seed that was sprouted, shrivelled, injured by frost, injured mechanically, or infected with pathogenic fungi. Each lot was divided into three parts. One of the parts was plated to determine the prevalence of infecting fungi, but particularly of *Helminthosporium sativum*, *H. teres*, and *H. Avenae.* The second part was subdivided into two, of which one was treated with diluted Ceresan and the other left untreated, and both were sown in a bed of non-sterile soil. The third part (non-disinfected) was germinated upon moist paper (paper towels) in the incubator at 75° F. and in a saturated atmosphere[1]. The results of the three tests were then summarized and correlated, as shown in Table VIII.

[1] *This part of the experiment was performed in the laboratory of the North West Line Elevators Association, Winnipeg, Man., through the courtesy of Dr. K. W. Neatby, Director of the Agricultural Department.*

TABLE VIII

COMPARISON OF THE PERCENTAGES OF SEEDS GERMINATING AND THE PERCENTAGES OF DISEASED SEEDS OR SEEDLINGS OBTAINED WITH WHEAT, OATS, AND BARLEY WHEN TESTED BY THREE DIFFERENT METHODS OF TESTING SEED

Factor studied	Kind of test	Seed treatment	Crop		
			Wheat	Oats	Barley
Percentage of seeds germinating	Soil (*a*)	Treated**	94.60	93.03	93.52
	Soil (*b*)	None	89.52	87.24	93.46
	Moist paper (*c*)	None	92.54	92.48	93.81
Percentage of diseased seeds or seedlings	Plating-out (*d*)	Treated***	3.16	4.94	8.20
	Soil (*e*)	None	3.49	15.99	7.53
	Moist paper (*f*)	None	38.43	2.07	8.17
Coefficients of correlation	Between (*a*) and (*b*)		0.566*	0.897*	0.891*
	Between (*b*) and (*c*)		0.545*	0.750*	0.665*
	Between (*d*) and (*e*)		0.628*	0.020	0.720*
	Between (*d*) and (*f*)		0.238	0.003	0.372*
	Between (*e*) and (*f*)		0.309*	0.215	0.363*

* *Denotes attainment of 1% level of significance.*

** *Dusted with dilute New Improved Ceresan.*

*** *Disinfected with mercuric-chloride–alcohol solution before being plated out.*

With respect to the percentage of seeds germinating, this percentage was highest when the seed was dusted with dilute Ceresan and then planted in non-sterile soil.. The percentage was lowest when non-dusted seed was planted in non-sterile soil. The coefficients of correlation for the percentages of seeds germinating show that germination on moist paper was more comparable to a soil test with disinfected seed than to a soil test with non-disinfected seed.

The percentage of infected seed or seedlings was found to vary with both the crop and the test. In wheat, the percentage of seeds found by the plating test to be infected with pathogenic fungi agreed closely with the percentage of lesioned seedlings from non-disinfected seed in non-sterile soil. The coefficient of correlation for these two tests was quite high (0.628). On moist paper, however, a large number of the wheat seedlings became infected secondarily through a spread of pathogenic fungi from infected seeds. On this account, the coefficient of correlation between the results of this test and those of the plating test was very low. The correlation between the moist paper test and the non-sterile soil test was also very low. In oats, the percentage of seedlings developing disease was greatest in non-sterile soil and least on moist paper. The percentage of seedlings showing disease in non-sterile soil was considered to be a more reliable index of the amount of seed infection by *Helminthosporium Avenae* than was the percentage of seeds found infected by the plating test. When the seed was planted in soil, practically every seedling found diseased showed a lesion typically produced by *H. Avenae.*

As this fungus apparently does not grow in non-sterile soil (10), the lesions evidently originated from infected seed. In contrast, the seedlings on moist paper failed to produce lesions typical of the disease. When the results of the three tests with oats were correlated, none of the coefficients of correlation were significant. In barley, as in wheat, there was close agreement between the results of the plating test and the test in non-sterile soil, while there was relatively little agreement of the results from either of these tests with those obtained on moist paper.

With respect to the different kinds of physical damage to the seed, experience showed that the test in non-sterile soil measured the amount of damage more accurately than did a test in plates of nutrient agar or a test on moist paper. Sprouted seed germinated better in soil than it did on moist paper. Many of the sprouted-seed lots, when dusted with dilute Ceresan and planted in soil, germinated surprisingly well. Shrivelled seed germinated as well on moist paper as in soil, but the difference between the diameters of the coleoptiles from shrivelled kernels and those from plump kernels was more pronounced in soil than on moist paper. The effect of frost injury to the seed was much more evident in the soil test than in the moist paper test. On moist paper, only severe frost injury produced any noticeable effect, while, in the soil test, even a slight degree of injury was readily evident. In soil, slight damage by frost to the seed resulted in a characteristic waviness of the veins near the tip of the primary leaf. With moderate damage, the entire primary leaf, sometimes also the second and third leaves, showed this waviness. With severe damage, the seed either failed to germinate or produced a crinkled, stunted plant.

Experiments with Barley Seed Infected with *Helminthosporium teres*

When barley seed, collected from various parts of Canada during the years 1938-1941, inclusive, was subjected to a plating test, it was found that infection of the seed with *Helminthosporium teres* was quite prevalent, the range of infection varying from 1 to 64%. However, when the infected seed was planted in beds of non-sterile soil, there was seldom any evidence of the disease in the seedlings. Severely infected seed lots produced a few plants with leaf lesions, but there was scarcely any basal lesioning. When infected seed was germinated at room temperature on moist paper in Petri dishes, spores of *H. teres* formed on the surface of the seed but no lesions developed on either the stems or leaves.

In view of the results obtained by Ravn (11), it was thought that the failure of the lesioning to appear was due to the relatively high temperature (20° C.) at which the seed was germinated in the soil. On this account, several modifications of the non-sterile soil method of testing seed were tried. In the first experiment, three lots of infected barley seed were planted in pots of non-sterile soil, the pots then being placed in a cool greenhouse (10° to 12.5° C.). They were kept at this temperature until the seedlings had

begun to emerge from the soil (eight days after planting). At this time, one-third of the pots were removed to a greenhouse kept at 20° C. Later, the remaining pots were removed to the warmer greenhouse, one-half of the remainder at the end of two days and the rest at the end of four days. The plants were examined when the third leaf had begun to show in the majority of plants in each set of pots. The results from four replicates of this test showed no difference whatever in the amount of leaf lesioning between the three sets of pots. There was a difference in the amount of leaf lesioning between seed lots, however, for two of the lots of seed (with 18% and 34% infection, respectively—determined by a plating test) showed only a trace of lesioning, while the third lot, with 49% of the seeds infected, produced 14% of plants with leaf lesions. There was scarcely any difference at all between sets of pots and between seed lots in the percentage of plants with basal lesions.

In a second experiment, the three seed lots just referred to were planted in autoclaved and in non-sterile soil, but otherwise the experimental procedure was the same as in the previous experiment. The results from four replicates of this experiment showed that there was very little difference in the amount of leaf lesioning produced in autoclaved and in non-sterile soil (10% and 11%, respectively). There was a significant difference, however, in the amount of basal lesioning. In autoclaved soil, 13.6% of the plants showed basal lesions while in non-sterile soil only 5.6% were thus affected.

In a third experiment, only one seed lot (49% infection with *H. teres*) was used, but the period of incubation in the cool greenhouse (10° to 12.5° C.) varied from 0 to 24 days. The seed was planted in quadruplicate sets of pots of non-sterile prepared soil to furnish four replicates of the test. Four pots from each set were placed in a greenhouse at 20° C., while the remaining pots were placed in the cool greenhouse mentioned above. At daily intervals afterwards until the seventh day, a group of 16 pots was removed to the warmer greenhouse, but one group of 16 pots was left in the cool greenhouse for 24 days. The plants were examined for leaf and basal lesions when the third leaf appeared. The results of the examination are summarized in Table IX. They show that, after the fourth day, there was a progressive increase in leaf lesioning with an increase in length of the incubation period at low temperature, and that there was no increase or decrease in the amount of basal lesioning.

In a fourth experiment with *Helminthosporium teres* a number of different lots of barley seed were planted in a large bed of non-sterile soil. The temperature of the greenhouse was kept at 10° to 12.5° C. for seven days after the seed was planted, and then raised to 20° C., at which temperature it was held for seven days. The percentages of seedlings with leaf lesions were determined at the end of the second seven day interval. Three further tests were made with the same seed and under the same conditions. The summarized results are given in Table X, and they show that the percentage of seedlings with leaf lesions is not necessarily related to the percentage of seeds

TABLE IX

RELATION OF DIFFERENT PERIODS OF INCUBATION AT 10° TO 12.5° C. ON THE PERCENTAGES OF SEEDLINGS WITH LEAF AND BASAL LESIONS IN A LOT OF BARLEY SEED SEVERELY INFECTED WITH *Helminthosporium teres*

Incubation period (days)	Percentage of seedlings with leaf lesions	Percentage of seedlings with basal lesions
0	2.25	3.50
1	3.00	4.50
2	5.00	4.25
3	2.75	2.50
4	6.00	1.50
5	9.75	2.25
6	12.75	3.00
7	15.25	3.50
24	20.50	2.00
Necessary difference (5% level)	4.80	3.52

that are infected. This seems to indicate that different seed lots of barley may be infected with different strains of *H. teres*. The difference between seed lots Nos. 14 and 15 is striking enough to suggest this possibility. Under such circumstances, a plating test with seed is obviously misleading. The experience of the writers, based on tests with hundreds of seed lots of barley, indicates that the proportion of barley seed lots infected with virulent strains of *H. teres* is relatively small.

A field experiment with infected barley seed was conducted in 1940. In this experiment, eight lots of barley seed, with an infection range from 0 to 34% were planted in rod rows on May 8 and the seedlings were examined one month later. The results were in accord with the greenhouse results shown in Table X. Only a trace of leaf lesioning appeared in the seed infection range from 0% to 15%, while in a seed lot with 34% of the seeds infected, 4.3% of the seedlings bore the reticulate lesions typically caused by the fungus. A second field experiment, made in 1941, gave a different result. In this, the percentages of seedlings with leaf lesions in the field agreed closely with the percentages of seeds found infected by the plating test, but when the same seed lots were planted in non-sterile soil under greenhouse conditions (20° C.), only one lot, with 25% of the seed infected, produced seedlings with lesions on the leaves.

These results of greenhouse and field experiments with barley seed indicate a variability in the pathogenicity of *Helminthosporium teres*, and also a relative unreliability of the non-sterile soil test for the appraisal of the amount of seed infection in barley by *H. teres*. There are indications, also, that the plating test for this fungus is also unreliable, as it apparently does not differentiate between virulent and non-virulent strains of *H. teres*. Tentatively,

TABLE X

RELATION OF THE PERCENTAGE OF SEEDS INFECTED WITH *Helminthosporium teres* TO THE PERCENTAGE OF SEEDLINGS WITH LEAF LESIONS WHEN THE SEEDLINGS WERE FIRST GROWN FOR SEVEN DAYS AT 10° TO 12.5° C. AND FOR AN ADDITIONAL SEVEN DAYS AT 20° C.

Seed lot number	Percentage of seeds infected with *H. teres*	Percentage of seedlings with leaf lesions
1	0	0.50
2	1	0.50
3	2	0.25
4	4	0.25
5	5	1.00
6	6	0.75
7	7	0.50
8	11	1.00
9	12	0.00
10	18	0.00
11	19	4.25
12	23	0.50
13	34	0.50
14	38	0.00
15	49	17.75
Necessary difference (5% level)		1.50

however, the plating test may be said to be the more accurate, because it does show when the fungus, whether innocuous or not, is present.

Recommendations Based on the Test in Non-Sterile Soil

Two distinct quantities have been measured by the non-sterile soil test for every sample that was tested, namely, (1) the percentage of seeds that was viable, and (2) the percentage of seeds producing healthy plants. From the farmer's view-point, the determination of the latter is probably the more important.

Following an examination for disease of several thousand samples of cereal seed (wheat, oats, and barley) in 1938, 1939, 1940, and 1941, it became evident that the samples could be classified into two principal groups, namely, samples in which the seed was free from smut or contained only a trace, and samples containing more than a trace of smut (covered smuts of wheat, oats, and barley, and loose smut of oats). The degree of infection by smut was determined by centrifuging the washings from the seed (5). These two principal groups of samples could be subdivided into two subgroups, namely, samples that germinated well either without or after seed treatment and samples that germinated poorly even after treatment. A further subdivision could be made on the basis of the degree to which seed disinfection increased the percentage of healthy plants. Within the main groups and subgroups just mentioned, there were several minor groups including (1) frosted seed, (2)

seed in which treatment increased the sturdiness of the seedlings without increasing their number, and (3) seed producing weak seedlings even after treatment.

On the basis of this grouping of seed samples, it was possible to set up a table of tentative recommendations regarding the handling of seed represented by the samples tested in non-sterile soil. These recommendations (Table XI) are based partly upon the results from unpublished field experiments made by the present writers and partly upon the information regarding smuts obtained from Mr. W. Popp, of this laboratory.

TABLE XI

RECOMMENDATIONS, BASED ON A CENTRIFUGE TEST FOR SMUT AND A SOIL TEST FOR PHYSICAL AND FUNGUS INJURY, REGARDING THE HANDLING OF CEREAL SEED BEFORE SEEDING

Smut spore load on seed	Percentage of healthy plants			Recommendations
	Treated seed	Non-treated seed	Increase from treatment	
Heavy trace to heavy††	91–100	*	*	Treat seed with organic mercury dust
	51–90	*	*	Treat seed and increase rate of seeding**
	0–50	*	*	Discard seed
	Frosted seed	*	*	See footnote***
None or trace	91–100	91–100	0–9	No treatment required
	91–100	0–90†	5 +	Treat seed with organic mercury dust
	51–90	51–90	0–5	Increase rate of seeding**
	51–90	51–85	5 +	Treat seed and increase rate of seeding
	0–50	—	—	Discard seed
	Frosted seed	—	—	See footnote***

* *On account of infection with smut, all seed should be treated.*

** *Percentage increase in rate of seeding* $= \frac{(100-x)}{(x)} \times 100$ *where* $x =$ *% of healthy plants in treated seed.*

*** *Ordinarily about 50% of frosted seeds produce normally growing plants. Theoretical percentage of healthy plants = % healthy seedlings* $+ \frac{\%\ frosted\ seedlings}{2}$. *Calculate increased seeding rate on basis of theoretical percentage.*

† *In barley, subtract percentage of seeds found by plating test to be infected with Helminthosporium teres from percentage of healthy seedlings from untreated seed.*

†† *Smut spore load exceeding 1 : 128,000.*

It is difficult to formulate recommendations equally applicable to areas with different climatic conditions. The results of field experiments (9) have shown, for instance, that in an area with semiarid climatic conditions, a low rate of seeding is likely to give a yield equal to that from a high rate of seeding, and have shown that, from seed treatment, moderate increases in density of stand fail to give any increase in yield. In such areas, therefore, low germinability of the seed, or the infection of the seed by seed-borne seedling blight fungi, seems to be of less consequence than in areas where the soil moisture is sufficient to support a dense population of plants. Similarly, the

writers found that, with seed-borne leaf-spotting diseases, infection may spread from the seed to the seedlings, but the relatively dry weather of summer, for example in Manitoba, prevents the disease from spreading in the field and doing harm. Under humid weather conditions, however, these diseases increase in destructiveness with the progress of the season. Thus, a high degree of seed infection by leaf spotting fungi may not reduce yields when the seed is sown under semiarid conditions, while even a trace of infection may be destructive when the seed is sown under humid conditions. Evidently, from these observations, seed of relatively low quality may produce a good crop in semiarid areas, while seed of only the best quality can do so in humid areas. This, however, should not be interpreted as suggesting that there should be different standards of seed quality for different areas, but only that, under certain conditions, seed with moderate germinability and a moderate amount of seed-borne disease may give yields equal to those from the best quality seed.

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SEARCH FOR SOURCES AND CARRIERS OF EQUINE ENCEPHALOMYELITIS VIRUS[1]

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Abstract

Brains of 112 ground squirrels (*Citellus richardsoni*), 10 hawks, one burrowing owl, three pheasants, four crows, nine rats (eight *Mus decumanus* and one *M. rattus*) two wild ducks, two jack rabbits, three hens, three pigs, and two cats were examined for the presence of virus. Spleens of 15 ground squirrels and livers of three others, also spleens of five hawks and six rats were examined. Six assassin bugs (one *Zelus audax* and five *Sinea diadema*), 133 mosquitoes, 30 *Stomoxys* sp., 10 *Tabanidae*, 30 crickets, 64 grasshoppers, and 12 ticks (*Dermacentor andersoni* Stiles) were also examined in suitable sized lots.

Two guinea pigs were injected with a suspension of each lot. If suspicious temperature reactions occurred, these animals were destroyed and suspensions of their brains were injected into other guinea pigs. All the injected animals that survived, regardless of whether or not they had shown any temperature reaction, were given a challenge inoculation of 25 M.L.D. of Western virus three weeks or longer after the first injection.

Lot 21, consisting of two ground squirrel brains, was the only group to give any evidence suggestive of the presence of virus. Virus could not be isolated from this lot but temperatures of the injected guinea pigs, and subsequent resistance of the survivor to a challenge inoculation, were very suggestive of its presence. This is the second occasion on which results suggested the presence of virus in very small amount in ground squirrel brains.

In 1940, Gwatkin and Moore (3) published a report indicating the possible presence of equine encephalomyelitis virus (Western) in a group of ground squirrel brains examined for the virus by guinea pig inoculation. A year later Gwatkin (2) reported negative results from the examination of 200 brains of ground squirrels, mice, and other small mammals and birds, 34 ground squirrel spleens, and 527 ticks (*Dermacentor andersoni* Stiles).

Cox *et al.* (1) recently isolated Western virus from a naturally infected prairie chicken during an epidemic of human encephalitis. Both the blood and central nervous system harboured the virus. Hammon *et al.* (5) reported the recovery of equine encephalomyelitis virus (Western) and St. Louis virus from mosquitoes (*Culex tarsalis*). They (4) carried out neutralization tests on the blood of wild and domestic animals and birds and consider it probable that the antibodies found in many of the species listed are the result of specific

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infection probably of a mild or unapparent nature. These results indicate that the search for carriers, while admittedly a very large undertaking, has definite justification for its pursuit.

The present report deals with the examination of material during the summer of 1941 for possible carriers of the virus. The procedure used formerly (2, 3) was adopted. Heads and organs of animals to be tested and insects were sent to the laboratory in jars of buffered glycerine solution which were iced for shipment. The brains were removed at the laboratory and placed in buffered glycerine solution. A portion of each brain was ground up with sterile alundum, the remainder being retained for future use if required; 0.2 cc. of each mixture suspended in 0.9% saline solution was injected, under ether anaesthesia, into two guinea pigs by the intracerebral route. In order to conserve guinea pigs, several brains or spleens or other organs from each group of animals to be tested were pooled to form each *lot*, the particulars of which are given below under their lot numbers. The temperature of each guinea pig was taken prior to injection and for at least one week afterwards. Those that showed temperature reactions were destroyed and a suspension of their brains was injected into two or more guinea pigs. All animals that survived for three weeks were given an intracerebral challenge inoculation of 25 M.L.D. (minimum lethal dose) of Western virus.

Lot 1: one engorged female of *Dermacentor andersoni* and 11 males, received from H. S. Seamans, Lethbridge, Alta., on May 14; picked off a horse (normal animal). They were ground up with sterile alundum and 0.2 cc. of the supernatant liquid was injected into two guinea pigs by the intracerebral route. The guinea pigs did not show any abnormal temperature reactions. They were given a challenge inoculation of virus by the same route three weeks later and both died.

Lot 2: brains of two ground squirrels collected by J. H. Brown in a flat west of Irvin, near Medicine Hat, Alta.; received May 26. Half of each brain was ground up and 0.2 cc. of brain suspension was injected into two guinea pigs by the intracerebral route. No abnormal temperature reactions appeared. The guinea pigs were challenged three weeks later, unfortunately with an insufficient dosage, as shown by the controls, and only one died. The remaining half of each brain was injected into two and three guinea pigs, respectively. These animals remained normal and died following challenge inoculation three weeks later.

Lot 3: brains of five ground squirrels, collected at the same place as Lot 2 by J. H. Brown and received the same day. This material was injected in the usual manner into two guinea pigs which, three weeks later, were challenged. They showed no abnormal temperature reactions before being challenged. Only one died, since the challenge dose, as shown by results in controls, was insufficient. To make certain of this, the halves of the five brains, which had been held in the refrigerator, were ground up and the usual injection of each was given to three guinea pigs. One of these 15 guinea pigs showed a temperature of 104.7° F. on the fifth day after injection. It was killed and

a suspension of its brain was injected into four new guinea pigs. The other 14 animals were challenged three weeks later and all died. On the eighth day one of the four guinea pigs had a temperature of 103.6° F. which, however, was reduced to 102.6° on the following day when the animal was killed and a suspension of its brain was injected into two additional guinea pigs. The other three were challenged at the usual time and died. One of the two guinea pigs remained normal; the other had a temperature of 103.8° F. on the fourth day. A suspension of its brain was injected into four guinea pigs all of which remained quite normal. It may therefore be concluded that there was no virus in the brains of the ground squirrels.

Lot 4: four ground squirrel heads, collected at the same place as Lots 2 and 3. Half of each brain was ground up and 0.2 cc. injected in the usual manner into two guinea pigs, the temperatures of which kept within a normal range. They were challenged three weeks later with virus and died following the usual chain of symptoms.

Lot 5: one assassin bug (*Zelus audax*) collected by Dr. R. Salt, Division of Entomology, Lethbridge; received June 21. It was washed in phenolized saline, ground up in 1 cc. of saline, and 0.2 cc. of the suspension was injected into each of two guinea pigs by the intracerebral route. Both animals remained normal as to health and temperature reactions. They were challenged three weeks later with virus and both died.

Lot 6: four ground squirrel brains collected by Mr. J. H. Brown 20 mi. north of Bassano, Alta., and received July 6. The owner of the premises on which these were taken reported the loss of one horse from encephalomyelitis in 1941 and, at the time, had one recovering. There were no human cases on the farm and no other livestock was affected. Ground squirrels were very scarce in this area. The brains were removed at the laboratory and half of each was ground up with saline and pooled. Two guinea pigs were given an intracerebral injection of 0.2 cc. of the suspension. One died two days later as the result of bacterial infection; the other was challenged three weeks after injection and died four days later.

Lot 7: three ground squirrel heads in buffer solution collected from the same place as Lot 6. A suspension of these brains was injected into two guinea pigs. They showed no abnormal temperature reactions and were given a challenge inoculation three weeks after the first injection. Both died five days later with typical symptoms.

Lot 8: one ground squirrel head from the same place as Lot 10. Brain suspension was injected into two guinea pigs and the temperatures were taken as usual. The animals were challenged three weeks later and both died.

Lot 9: livers containing parasites; taken from two ground squirrels which were from the same place as Lot 6. Portions of liver were ground up and the suspension was injected in the usual way into two guinea pigs. Neither animal showed abnormal temperature reactions. They were challenged three weeks later and both died.

Lot 10: four ground squirrel heads collected by Mr. J. H. Brown from a pasture one mile north and one mile west of Countess, Alta. One horse on the premises was recovering from equine encephalomyelitis. There had been no such cases in 1940 and no human cases. The ground squirrel population was reported as normal in number. A suspension of the brains was injected into two guinea pigs without producing any abnormal reaction. They were challenged three weeks later and both died.

Lot 11: two hawk heads from the same place as Lot 10. Brain suspension was injected into guinea pigs by the intracerebral route. Temperatures remained normal. Challenge three weeks later resulted in death.

Lot 12: six ground squirrel heads collected by Mr. J. H. Brown three miles north and one mile west of Countess. The owner reported two cases of equine encephalomyelitis in 1941, five cases in 1938, and two in 1936, none of which were fatal. Of the two horses affected in 1941 one had recovered at the time the report was made. The owner had been affected in 1938 with a disease which he believed he had contracted from the horses. Ground squirrels were reported as having decreased in 1939 and 1940. Brain suspension was injected in the usual manner into two guinea pigs. One died the following day from bacterial infection. The other lived and was challenged three weeks later. It died five days after the challenge inoculation.

Lot 13: head of a burrowing owl, from the same place as Lot 12. This was treated in the usual manner. There were no temperature reactions and both guinea pigs died following challenge inoculations three weeks after injection.

Lot 14: brains of two ground squirrels from the same place as Lot 12. Injection of brain suspension did not give rise to any temperature reactions and both animals died following challenge inoculations three weeks later.

Lot 15: one ground squirrel brain from the same location as Lot 12. A suspension of brain was injected into two guinea pigs by the usual method. One guinea pig did not show any temperature reaction but the other, commencing the following day, had daily temperatures of 105.6°, 101.6°, 105.2°, 105.0°, 104.0°, and 104.0° F. It was killed on the sixth day, the brain was cultured, and the usual brain suspension was injected into two more guinea pigs. These showed no reaction. The original survivor and the second two animals were given a challenge inoculation three weeks after the first injection and all died. The sudden rise of temperature is not typical of virus symptoms but the brain culture showed only one bacterial (coccus) colony. However, the absence of resistance in the challenged animals proves the absence of virus.

Lot 16: brain of jack rabbit, collected by Rocky Mounted Spotted Fever and Sylvatic Plague Survey at the same place as Lot 6 on Aug. 6. A suspension of brain was injected into two guinea pigs. These animals showed no temperature reaction and died following challenge inoculation three weeks later.

Lot 17: this consisted of four ground squirrel heads collected by R.M.S.F. and S.P. Survey on Aug. 7. The brains were suspended in saline and 0.2 cc. was injected into two guinea pigs. One was dead the following day. The other animal did not manifest any unusual temperature reaction. It was challenged three weeks later and died on the fifth day.

Lot 18: six ground squirrel heads collected by R.M.S.F. and S.P. Survey two miles north of Countess on Aug. 7. The owner reported three cases of equine encephalomyelitis in 1941; all of the animals were recovering at the time. One horse was affected in 1936 and one in 1937; both recovered. No humans or other animals were affected. The ground squirrel population had decreased in 1940 and 1941. The owner further reported that some of his chickens had a similar condition in 1938 but as adult fowl do not show symptoms following artificial infection, and paralyses due to other causes are common among them, this is not necessarily important. An injection of 0.2 cc. of pooled brain suspension was given to two guinea pigs. They remained in good health, showing no temperature reaction, and died following challenge inoculation three weeks later.

Lot 19: one ground squirrel head, collected by R.M.S.F. and S.P. Survey on Aug. 7 at the same place as Lot 10. This animal appeared quite ill and was dragging itself around by its forelegs. A suspension of brain was injected into two guinea pigs. One died five days after injection having had a temperature of 105.0° F. from the day after injection. The brain when cultured yielded a heavy growth of Gram negative rods. The other guinea pig ran a high temperature for a few days but the temperature was down to normal within a week. The animal died 17 days after injection. There was no evidence of peritonitis and culture from the brain was sterile. The temperature was probably due to bacterial infection, as when caused by virus it does not appear for several days.

Lot 20: head of a hen which was found dead on Aug. 7 on farm three miles north and four miles east of Duchess, Alta., collected by the R.M.S.F. and S.P. Survey. There were no sick horses on this farm but there was one on a farm two miles south and two miles west. On Aug. 6 a resident of the first farm was admitted to hospital with what was diagnosed as encephalitis. (Blood sample from this man was examined by the authors on Sept. 15 at request of the physician and was positive.) Two guinea pigs were injected with brain suspension. They showed no abnormal reaction and were challenged three weeks after the injection. Both died five days after the challenge inoculation.

Lot 21: two ground squirrel heads collected by R.M.S.F. and S.P. Survey one mile east and one mile north of Gem, Alta., on Aug. 8. The owner reported a registered Percheron stallion affected with encephalomyelitis. He had one case in 1938. He also reported a considerable decrease in the ground squirrel population in 1940 and 1941. He had lost some chickens from no apparent cause. The brains were ground up and 0.2 cc. of suspension was

injected in the usual manner into two guinea pigs. Temperatures of these animals are shown in Table I.

TABLE I

TEMPERATURES OF GUINEA PIGS INJECTED WITH SUSPENSIONS OF GROUND SQUIRREL BRAINS (LOT 21)

Guinea pig No.	Days after injection							
	0	1	2	3	4	5	6	7
	Temperature, °F.							
31	101.8	103.2	101.4	102.4	101.8	104.6	102.4	Killed
32	102.0	104.0	100.8	102.8	102.2	103.3	101.8	101.4

On the fifth day, No. 31 had a temperature of 104.6° F. which was down to 102.4° F. the following day, when the animal was killed. No. 32 showed some temperature rise on the fifth day but was allowed to live. When challenged three weeks later, together with 29 other injected guinea pigs and two controls, it survived the challenging dose of 25 M.L.D. which proved fatal for all the others.

Brain of No. 31 was ground up and injected into two more guinea pigs, the temperatures of which are shown in Table II.

TABLE II

TEMPERATURES OF GUINEA PIGS INJECTED WITH BRAIN SUSPENSION OF GUINEA PIG NO. 31

Guinea pig No.	Days after injection							
	0	1	2	3	4	5	6	7
	Temperature, °F.							
84	103.5	102.2	102.0	101.4	102.2	101.4	101.8	102.0
85	102.5	102.7	102.0	101.5	101.2	102.0	101.5	101.6

Nos. 84 and 85 did not show any abnormal temperature following injection. They were challenged with 25 M.L.D. of virus three weeks later and both died.

The original material was injected on Aug. 9 and, owing to the usual period of waiting to challenge, it was not again injected until Sept. 4. At that time the remaining portion of each brain was ground up and injected into two guinea pigs. These are described as Lots 21*A* and *B*. Temperatures of injected guinea pigs are shown in Table III.

TABLE III

TEMPERATURES OF GUINEA PIGS INJECTED WITH BRAIN SUSPENSIONS OF LOTS 21*A* AND *B*

Lot	Guinea pig No.	Days after injection							
		0	1	2	3	4	5	6	7
		Temperature, °F.							
21*A*	124	102.0	102.8	101.2	101.2	102.8	102.8	102.0	101.2
	125	101.2	102.7	103.4	Killed				
21*B*	126	100.0	102.4	101.2	102.0	103.0	103.0	102.0	101.0
	127	102.7	103.0	100.4	101.8	102.0	102.0	101.0	101.6

There was a marked haemorrhage in the brain of No. 125. Cultures showed only a few colonies of bacteria. This brain was ground up and injected into two guinea pigs, temperatures of which are given in Table IV.

TABLE IV

TEMPERATURES OF GUINEA PIGS INJECTED WITH BRAIN SUSPENSION OF GUINEA PIG NO. 125

Guinea pig No.	Days after injection							
	0	1	2	3	4	5	6	7
	Temperature, °F.							
243	102.0	102.0	101.4	101.8	102.4	101.4	101.4	101.8
244	101.0	101.6	101.2	102.3	102.3	102.6	102.0	102.2

Nos. 243 and 244 were challenged with the usual dose of virus three weeks later and both died. Nos. 124, 126, and 127 also died following challenge.

A repeat injection of suspensions of 21*A* and *B* was made six days after that in Table III. Temperatures of these animals are shown in Table V.

TABLE V

TEMPERATURES OF GUINEA PIGS INJECTED WITH SIX-DAY SUSPENSIONS OF LOTS 21*A* AND *B*

Lot	Guinea pig No.	Days after injection							
		0	1	2	3	4	5	6	7
		Temperature, °F.							
21*A*	239	101.0	104.0	101.8	101.0	101.0	101.0	101.0	100.5
	240	101.5	104.4	100.6	101.2	101.8	100.4	101.0	101.6
21*B*	241	100.4	103.0	101.4	101.8	102.4	101.4	101.4	101.8
	242	101.0	101.6	101.2	102.3	102.3	102.6	102.0	102.2

Guinea pigs Nos. 239 to 242 did not show any reaction from the injections. They were challenged at the usual time and all died.

It is believed that the temperature reaction of guinea pig No. 31 taken in conjunction with the immunity that developed in No. 32 may indicate the presence of a trace of virus in the ground squirrel brains. It was not possible, however, to reproduce the immunity in other guinea pigs as was done by Gwatkin and Moore (3). Therefore there is less certainty in the present case that the immunity in guinea pig No. 32 was due to virus in the original ground squirrel brains, but the results suggest this possibility.

Lot 22: six ground squirrel brains collected by the R.M.S.F. and S.P. Survey on Aug. 8 on rangeland four miles south and four miles west of Gem. The owner reported seven cases of the disease in 1941, two of which were fatal. In 1940 he lost one horse and in 1938 he had many cases, 20 of which were fatal. No domestic animals on the premises were ill in 1941 but in 1940 he lost a number of chickens from what appeared to be some brain disease. Ground squirrels were quite scarce. The brains of those collected were ground up and the suspension was injected into two guinea pigs. One had a temperature of 104.7° F. on the fourth day after injection and was killed. The brain yielded a heavy growth of bacteria and was discarded. The other animal did not give a temperature response and died five days after challenge inoculation at the usual time.

Lot 23: one ground squirrel brain collected by the Survey on Aug. 9 on a farm three and one-half miles north and four miles west of Crowfoot. At the time, the owner reported two sick horses, a mare and a colt; both were recovering. In 1938 and 1940 two horses were affected each year. All recovered. In 1941 he lost a number of chickens from no apparent cause. The ground squirrel population was normal in number but was ill in the spring. In 1938, also, the owner had noticed that the ground squirrels were ill, dragging themselves along by their forelegs and appearing to stagger. Guinea pigs were injected as usual with brain suspension and remained normal. They died following challenge inoculation three weeks later.

Lot 24: five ground squirrel heads from same place as Lot 23. Guinea pigs injected in the usual manner died from bacterial infection.

Lot 25: two ground squirrel heads from same place as Lot 23. Injected guinea pigs died from bacterial infection.

Lot 26: two ground squirrel heads from same place as Lot 23. Injected guinea pigs both ran high temperatures but as the squirrel heads were in the same shipment as Lots 24 and 25, it was believed that the reaction was due to bacterial infection. This was confirmed by challenge inoculation three weeks later, at which time both guinea pigs died.

Lot 27: portion of liver of ground squirrel from same place as Lot 23. Intracerebral injection in guinea pigs resulted in death of these animals from bacterial infection.

Lots 28 and 29: each lot consisted of one cat brain from Rose Lynn and Hanna, Alta. Both lots were in bad condition and injected guinea pigs died from bacterial infection.

Lot 30: one chicken head collected by the Survey on Aug. 14 at a farm near Watts. The owner had lost 17 chickens and some pigs in 1941 and some chickens in 1940. In 1938, three colts and one 12-yr. old horse suffered from encephalomyelitis. In 1940 the ground squirrels became scabby and a number died. Injected guinea pigs showed no temperature reactions and died following challenge inoculation three weeks later.

Lot 31: two pig brains from the same place as Lot 30. These pigs were three weeks old. One had died and the other was quite ill. Examination disclosed a spotted spleen and haemorrhagic intestines. "They appeared to be suffering from haemorrhagic septicaemia". A suspension of this material was injected into guinea pigs, which remained well and were challenged three weeks later, dying five days after the challenge inoculation.

Lot 32: one head of domestic chicken collected by the Survey at a farm near Hanna. The bird was paralysed and had been ill four days. Twelve had died in the same manner. The owner had also lost about 45 spring turkeys. Twenty chickens had been lost in 1938. No horses were sick. The ground squirrels were said to be mangy. A suspension of this brain was injected into two guinea pigs, one of which was dead the following day from bacterial infection. The other was challenged three weeks later and was dead on the fifth day after the challenge inoculation.

Lot 33: brains of three ground squirrels collected by the Survey on a farm near Rose Lynn adjacent to a pasture where a filly developed encephalomyelitis. (No ground squirrels were seen in this pasture.) Before the horse took sick a pig became paralysed and died. Ground squirrels were on the decrease in 1940 and 1941. A suspension of the brains was injected into two guinea pigs. One died the following day from bacterial infection. The other showed some temperature reaction for a few days and then returned to normal. It died following a challenge inoculation three weeks later.

Lot 34: six ground squirrel heads collected by the Survey near Rose Lynn on Aug. 17. The owner reported having lost one horse in 1941 from encephalomyelitis and 25 chickens from range paralysis. No other domestic animals were affected. The ground squirrel population was apparently normal in number. In 1938 he lost three colts. A suspension of these brains was injected in the usual manner into two guinea pigs. One was dead the following day and the brain yielded a heavy growth of bacteria. The other survived and, three weeks later, was given a challenge inoculation to which it succumbed.

Lot 35: five ground squirrel heads from the same shipment as Lot 34. Two heads were discarded owing to injury from shot. The remaining three were ground up and injected into two guinea pigs but both animals were dead the following day from bacterial infection.

Lot 36: four ground squirrel heads collected by the Survey, Aug. 29, on a farm near Enchant, Alta. Two horses had been affected with encephalomyelitis in 1941 and one of them died. The brains were ground up and injected in the usual manner into two guinea pigs. These animals showed no abnormal reaction and died following challenge inoculation three weeks later.

Lot 37: one jack rabbit head. This animal was collected by the Survey at the same place as Lot 36. Guinea pigs were injected with the brain and challenged three weeks later, following which both died.

Lot 38: seven ground squirrel heads from a farm near Enchant collected by the Survey on Aug. 29. Two horses came down with encephalomyelitis in the pasture in which these were collected. On a farm adjacent to this a woman died of encephalitis. Ground squirrels were very plentiful. Three heads in this lot had to be discarded on account of injury which made them unsuitable for injection. The others were ground up and injected into two guinea pigs. These animals did not show any temperature reaction and died following challenge inoculation of virus three weeks later.

Lot 39: five assassin bugs (*Sinea diadema*) collected by H. L. Seamans in the Swift Current–Vanguard district in Saskatchewan. They were washed in phenolized saline, ground up, and injected into two guinea pigs. One of these animals was dead the following day and was replaced with another, which also died. The survivor had a temperature of 105° F. on the second day after injection but returned to normal. It died following challenge three weeks later. Loss of the two guinea pigs was due apparently to some toxic property.

This material was held for a time to see if the toxic quality would disappear. The suspension was then filtered through a Berkefeld candle (V). Growth occurred in the filtrate so it was refiltered, after which it proved sterile. Two guinea pigs were injected by the intracerebral route with 0.2 cc. each. Both were dead the following morning.

Only one of the five guinea pigs injected with this lot survived but, as shown above, it did not show any evidence of the presence of virus.

Lot 41: five mosquitoes collected at the Klinck farm, Yellowgrass, Sask., on Sept. 4. They were washed in phenolized saline, ground up, and injected into two guinea pigs. These animals showed no temperature response and died following inoculation of virus three weeks later. Lots 41 to 76 were collected in Saskatchewan in connection with the investigation concerning encephalitis.

Lot 42: thirty mosquitoes collected at Weyburn, Sask. and preserved in buffer solution. They were washed in phenolized saline, ground up, and 0.2 cc. of suspension was injected into two guinea pigs by the intracerebral route. The injected guinea pigs showed no temperature response and died following the usual challenge inoculation of 25 M.L.D. of virus three weeks later.

NOTE: *Lot 40 was human spinal fluid; results not reported here.*

Lot 43: thirty-three mosquitoes collected at Weyburn, Sask. They were handled as Lot 42. Guinea pigs remained well and died following the challenge inoculation.

Lot 44: thirty-five mosquitoes collected at Weyburn. The injected guinea pigs remained well and died following challenge three weeks later.

Lot 45: thirty mosquitoes collected at Weyburn. Injected guinea pigs remained well and died following the challenge inoculation three weeks later.

Lot 46: spleen of hawk collected 10 mi. west of Weyburn. A portion of the spleen was ground up with alundum and injected by the intracerebral route into two guinea pigs. One of these was dead the following day. The survivor showed no temperature reaction and died following the challenge inoculation three weeks later.

Lot 47: four ground squirrel heads collected in the vicinity of Ceylon on Sept. 1. They were treated in the usual manner. The two injected guinea pigs showed no temperature reaction and died following the usual challenge inoculation.

Lot 48: two hawk heads collected 10 mi. west of Weyburn on Sept. 1. Suspension of brains was injected into two guinea pigs. No temperature reactions occurred and the guinea pigs died following the challenge inoculation three weeks later.

Lot 49: two duck heads collected at Kleinsaucer Lake on Sept. 1. Autopsy of these animals suggested the occurrence of Western duck sickness. Moreover, ducks were sick on this lake which is very shallow and of the type in which duck sickness occurs; not a great number of dead ducks were seen however—perhaps 25. Suspensions of the brains were injected in the usual manner into guinea pigs, which showed no temperature reactions. They died following challenge inoculation three weeks later.

Lot 50: two ground squirrel heads collected at the Klinck farm near Yellowgrass. Injected guinea pigs showed no reaction to the injection of brain suspension and died following the usual challenge inoculation three weeks later.

Lot 51: two ground squirrel spleens from the Klinck farm, collected Aug. 31. These were ground up and injected in the usual manner. The injected guinea pigs showed no reaction and died following the challenge inoculation three weeks later.

Lot 52: brain of hawk found dead at Kleinsaucer Lake. Injected guinea pigs remained well and died following challenge inoculation three weeks later.

Lot 53: six ground squirrel heads collected five miles northeast of Weyburn on Sept. 2. A suspension of these brains was injected into two guinea pigs in the usual manner. These animals gave temperature reactions which are shown in Table VI.

No. 156 was killed on the fourth day after injection when the temperature was 105.6° F. The brain was removed, examined microscopically, and cultured. Many gram negative rods were seen on direct smears and cultures

TABLE VI

TEMPERATURES OF GUINEA PIGS INJECTED WITH SUSPENSION OF GROUND SQUIRREL BRAINS

Guinea pig No.	Days after injection									
	0	1	2	3	4	5	6	7	8	
	Temperature, °F.									
155	100.2	101.6	101.8	101.2	102.0	103.5	103.0	103.2	104.2	Killed
156	100.3	102.0	103.0	105.8	105.6	Killed				

yielded a heavy growth of the same organism. No. 155 was killed on the eighth day when the temperature was 104.2° F. The brain yielded only one colony of bacteria and was injected into two guinea pigs three days after removal. The temperatures of these animals are given in Table VII.

TABLE VII

TEMPERATURES OF GUINEA PIGS INJECTED WITH BRAIN SUSPENSIONS OF No. 155

Guinea pig No.	Days after injection							
	0	1	2	3	4	5	6	7
	Temperature, °F.							
270	100.0	101.4	101.6	101.2	101.2	101.0	101.6	101.6
271	101.2	102.0	102.6	102.3	102.0	101.8	102.8	102.0

Nos. 270 and 271 did not show any abnormal temperature reaction and remained in good health. They died following challenge inoculation three weeks later so it may be assumed that the original temperature reactions were due to bacterial infection.

Lot 54: five ground squirrel heads collected five miles northeast of Weyburn on Sept. 2. The brains were ground up, pooled, and injected into two guinea pigs. These animals showed no temperature reaction and died following a challenge inoculation three weeks later.

Lot 55: four spleens from ground squirrels (Lot 54). Injected guinea pigs showed no reaction and died following challenge inoculation three weeks later.

Lot 56: three ground squirrel spleens from the same place as Lot 54. The injected guinea pigs gave no reaction following injections of spleen and died after a challenge inoculation three weeks later.

Lot 57: three hawk heads, collected on highway No. 13 near Hume, Sask. Guinea pigs injected with brain suspension remained normal and died following challenge inoculation three weeks later.

Lot 58: four hawk spleens. Guinea pigs injected with the material showed no temperature reaction. They died following challenge inoculation three weeks later.

Lot 59: two hawk heads and one ground squirrel head, collected on highway No. 13, near Hume, on Sept. 3. There was no reaction following injection of this material and the guinea pigs died after a challenge inoculation three weeks later.

Lot 60: four ground squirrel heads, collected on Sept. 4, three miles southwest of Hume. The injected guinea pigs did not show any reaction and died following the subsequent challenge inoculation.

Lot 61: four ground squirrel heads, collected Sept. 4, at a point three miles southwest of Hume. One head had been injured and was discarded. The other brains were ground up and injected into two guinea pigs. One of these animals showed a slight temperature rise on the eighth day after injection and was killed and the brain removed. Temperatures are shown in Table VIII.

TABLE VIII

TEMPERATURES OF GUINEA PIGS INJECTED WITH GROUND SQUIRREL HEADS (LOT 61)

Guinea pig No.	Days after injection									
	0	1	2	3	4	5	6	7	8	
	Temperature, °F.									
171	99.0	102.0	101.8	101.4	101.4	101.0	101.8	101.8	101.0	
172	100.4	103.0	103.0	102.6	102.0	102.0	102.0	103.5	103.6	Killed

No. 172 was killed on the eighth day after injection as it was showing some increase in temperature. The brain was ground up and injected into another two guinea pigs, temperatures of which are shown in Table IX.

TABLE IX

TEMPERATURES OF GUINEA PIGS INJECTED WITH BRAIN SUSPENSION OF GUINEA PIG NO. 172

Guinea pig No.	Days after injection							
	0	1	2	3	4	5	6	7
	Temperature, °F.							
272	100.0	101.6	103.4	102.5	102.2	102.4	102.0	101.6
273	101.5	101.8	102.0	101.6	102.2	101.6	101.8	102.4

Guinea pigs Nos. 272 and 273 showed no reaction and died following challenge injection three weeks after the first injection. No. 171 was also challenged three weeks after the first injection and died with the usual train of symptoms.

Lot 62: six ground squirrel spleens collected Sept. 4 at a point three miles southwest of Hume. A portion of each spleen was ground up together and 0.2 cc. of suspension injected into two guinea pigs. Temperatures of these animals are shown in Table X.

TABLE X

TEMPERATURES OF GUINEA PIGS INJECTED WITH SUSPENSIONS OF SPLEENS (LOT 62)

Guinea pig No.	Days after injection									
	0	1	2	3	4	5	6	7	8	
	Temperature, °F.									
173	101.8	101.4	100.8	101.0	101.4	101.6	101.8	103.6	103.8	Killed
174	100.4	101.8	101.8	100.6	101.2	101.2	103.8	103.7	99.0	Killed

Both guinea pigs showed a temperature rise on the seventh day after injection. Both animals were killed on the eighth day, the brains removed, and cultured. At this time No. 174 was ill and its temperature was going down. There was evidence of peritonitis in No. 174 but not in No. 173. Brain cultures of both were negative. Blood of No. 173 was collected for neutralization test, the result of which was questionable. Peritoneal cultures of No. 173 were negative but No. 174 yielded a heavy growth of bacteria.

The brain of No. 173 was ground up and injected into guinea pigs, Nos. 274 and 275, two days after removal. Temperatures of these animals are given in Table XI.

TABLE XI

TEMPERATURES OF GUINEA PIGS INJECTED WITH BRAIN SUSPENSION OF GUINEA PIG NO. 173

Guinea pig No.	Days after injection							
	0	1	2	3	4	5	6	7
	Temperature, °F.							
274	100.6	101.5	102.6	102.0	101.7	101.8	102.0	101.4
275	101.0	101.2	103.4	101.8	102.7	102.8	102.6	102.4

Nos. 274 and 275 remained well and died following the usual challenge inoculation. Nothing was done with brain of No. 174 as death of this animal was due to peritonitis.

Lot 63: thirty stable flies (*Stomoxys* sp.) collected near Trossachs on the highway on Sept. 1. They were washed, ground up, and injected into guinea pigs in the usual manner. One guinea pig showed a marked temperature rise on the second day after injection (105.8° F.) the other, a temperature of 103.8° F. on the same day. Both rapidly returned to normal. Because of its early appearance the reaction was ascribed to bacterial infection, or toxic property of the flies, and this was confirmed by challenge inoculation of these animals three weeks later, following which both animals died.

Lot 64: nine specimens of *Chrysops* sp. and one of *Tabanus* sp. collected Sept. 1 on the highway near Trossachs. These were washed, ground up, and injected into two guinea pigs, which showed no reaction. They died following subsequent challenge inoculation.

Lot 65: four rat heads (*Mus decumanus*) collected at Weyburn waste disposal ground. They were injected in the usual way into two guinea pigs, which did not show any disturbance and which died following the usual challenge three weeks later.

Lot 66: four rat heads (*M. decumanus*) from same source as Lot 65. Guinea pigs were injected in the usual manner. Temperatures of these animals are shown in Table XII.

TABLE XII

TEMPERATURES OF GUINEA PIGS INJECTED WITH RAT BRAINS (LOT 66)

Guinea pig No.	Days after injection							
	0	1	2	3	4	5	6	7
	Temperature, °F.							
183	100.8	102.0	105.0	104.0	104.4	102.4	103.0	102.2
184	102.0	102.2	104.6	102.2	101.0	101.8	102.4	102.2

No. 183 died 21 days after injection. Death was due to peritonitis and the brain was discarded. No. 184 was challenged about three weeks after the first injection and died five days later.

Lot 67: one black rat head (*Mus rattus?*) collected at the same place and time as Lots 65 and 66. Both guinea pigs injected with brain suspension remained well, showed no temperature reaction and died following subsequent challenge inoculation.

Lot 68: one brain of domestic pig which died near Weyburn. Autopsy was conducted at the request of Dr. A. J. Andries but, owing to decomposition of the carcass, was not satisfactory. The brain was collected and a suspension prepared and injected into guinea pigs in the usual manner. The brain must have been cleaner than the carcass for the injected guinea pigs did not show any reaction. They died following the usual challenge inoculation.

Lot 69: six rat spleens removed from some of the rats collected at Weyburn. They were ground up and injected in the usual manner into two guinea pigs, which remained well and died following challenge inoculation.

Lot 70: fifteen black crickets collected near Lang around a house where a patient had encephalitis. Two guinea pigs injected with suspension were dead the following morning as a result of infection.

Lot 71: twelve black and three light coloured crickets collected in the same place as Lot 70. One of the injected guinea pigs survived the injection but showed an immediate rise of temperature. This animal died following subsequent challenge inoculation.

Lot 72: twelve grasshoppers collected between the Klinck farm and Weyburn. They were washed in phenolized saline, as was done with all the insects, ground up with sterile alundum, and the usual injection made into two guinea pigs. These animals showed no abnormal temperature reaction and died following injection of the challenge dose three weeks later.

Lot 73: twelve grasshoppers from the same place as Lot 72. Guinea pigs were injected in the same manner but both died the following day, owing to bacterial infection.

Lot 74: forty grasshoppers, from the same source as Lot 73, were ground up with alundum to make a saline suspension. This was filtered through paper pulp and a Berkefeld candle (V) and 0.2 cc. was injected into each of two guinea pigs. These animals did not show any abnormal reaction and both died following the usual challenge inoculation.

Lot 75: this consisted of suspensions of Lots 70 and 71, pooled and filtered through paper pulp and a Berkefeld candle (V). This was injected into two guinea pigs by the intracerebral route. One of these animals, No. 266, died 11 days after injection. The brain showed the presence of gram positive rods which did not grow on aerobic culture. The other animal, No. 267, was given a challenge inoculation three weeks later and died with the usual train of symptoms.

Lot 76: this consisted of filtrate of Lot 39 and has been reported under that number.

Lot 77: three pheasant heads received Sept. 13 from Mr. G. Allen Mail of Kamloops. They were shot less than two miles from a farm where two horses were down with encephalomyelitis the previous month. Two guinea pigs were injected in the usual manner with the suspension and showed no reaction. They both died following challenge inoculation.

Lot 78: heads of four crows from Mr. G. Allen Mail, Kamloops, shot on the laboratory property where one horse died in 1940 of encephalomyelitis. Two guinea pigs were injected with brain suspension. They remained well and died following the subsequent challenge inoculation three weeks later.

Discussion

Lot 21, consisting of two ground squirrel brains, was the only group to show any evidence suggestive of the presence of virus. Both guinea pigs injected with a suspension of these brains gave a temperature reaction. One was killed and a suspension of its brain was injected into other guinea pigs, with negative results. The surviving guinea pig of the original pair resisted a subsequent challenge inoculation, which proved fatal for the two controls and 29 other guinea pigs injected at the same time.

The temperature reactions and resistance to challenge of the surviving guinea pig suggests the presence of a trace of virus in the ground squirrel brains. Unfortunately, by the time the challenge results were known the original material had been held for 26 days, which would result in an even lower concentration of virus, if such were present, than at the time of the first injection.

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SOME RECENT BIRD RECORDS FROM CANADA'S EASTERN ARCTIC[1]

By T. M. Shortt[2] and H. S. Peters[3]

Abstract

The list includes 82 species of birds from Canada's eastern Arctic. Opportunity for field observation was afforded the authors, separately, during two voyages of the *Nascopie* (1938 and 1939). Annotations concern numbers, dates of occurrences, habits, and distribution. Notable range extensions are recorded. Taxonomic notes are based on comparisons of a collection secured in 1938 and on additional unreported material recently acquired from the region by the Division of Birds, Royal Ontario Museum of Zoology.

Since 1933 the R.M.S. *Nascopie* has carried the Canadian Government Arctic Patrol on its annual visit to the eastern Arctic. The personnel is composed chiefly of administrators, medical officers, and scientists whose purposes are to supervise the health and general welfare of the natives and to make scientific investigation of the region.

The following annotated list of birds is based primarily on the authors' observations made while attached to the Government party on board the *Nascopie* in 1938 (T.M.S.) and 1939 (H.S.P.). On the 1938 trip, 173 bird specimens of 44 species were preserved. In addition, information derived from recent collections which have been made in the region and which have been acquired by the Royal Ontario Museum of Zoology have been included. These collections consist of: 109 skins from Somerset Island, collected by L. A. Learmonth of the Hudson's Bay Company in 1938–40; 59 birds taken at Lake Harbour, Baffin Island, by Rev. C. L. W. Bailey in the years 1930–32; and a few specimens secured by Rev. D. B. Marsh at Eskimo Point, N.W.T., approximately 160 mi. north of Churchill, Man.

The list is not intended as a catalogue of the birds of the eastern Arctic. It places on record a number of range extensions and new occurrences. Records for the two years are given separately in the text.

For the sake of brevity, dates have been omitted except where essential. However, the itinerary of the *Nascopie's* cruises for 1938 and 1939 has been included (Table I and Fig. 1) as an index to time and place.

Annotated List

Gavia immer (Brünnich). Common Loon.—*1938:* Two adults were noted on a small lake near Glasgow Falls, Lake Harbour, southern Baffin Island on July 20 and one individual was observed at Sugluk West, Que. At Pangnirtung, Baffin Island, skins of this species were seen in mats made by Eskimos. Information to the effect that these birds had been taken locally was secured from the Royal Canadian Mounted Police. *1939:* Five were seen a few miles east of Greenly Island in Belle Isle Strait on July 11.

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TABLE I

ITINERARY OF THE R.M.S. *Nascopie* IN 1938 AND 1939

Port of call	1938		1939	
	Arrived	Departed	Arrived	Departed
Montreal, Que.	—	July 9	—	July 8
Hebron, Labrador	July 15	16	July 17	18
Port Burwell, N.W.T.	17	18	19	20
Lake Harbour, N.W.T.	20	21	22	23
Wakeham Bay, Que.	21	22	24	25
Sugluk West, Que.	22	23	25	26
Cape Dorset, N.W.T.	24	26	27	28
Wolstenholme, Que.	26	27	28	29
Southampton I., N.W.T.	28	29	30	31
Cape Smith, N.W.T.	30	31	Aug. 1	Aug. 1
Port Harrison, Que.	Aug. 2	Aug. 4	2	4
Churchill, Man.	6	8	6	8
Chesterfield, N.W.T.	10	12	10	12
Wolstenholme, Que.	13	14	13	14
Lake Harbour, N.W.T.	16	17	15	15
Port Burwell, N.W.T.	18	19	16	16
Thule, Greenland	24	25	—	—
Craig Harbour, N.W.T.	26	26	22	24
Arctic Bay, N.W.T.	28	29	Sept. 3	Sept. 4
Fort Ross, N.W.T.	31	Sept. 1	Aug. 29	Aug. 31
Pond Inlet, N.W.T.	Sept. 3	4	Sept. 5	Sept. 6
Clyde River, N.W.T.	5	6	7	8
Pangnirtung, N.W.T.	9	12	12	15
Hebron, Labrador	14	15	18	19
Halifax, N.S.	19	Voyage ends	23	Voyage ends

Six specimens of the common loon were received in the collections made by Bailey. These had been taken at Lake Harbour and Saddleback Island, Baffin Island, at various dates in June and July, 1930 and 1932. The fact that these birds are not sexed, together with a lack of sufficient comparative material, makes it unwise to venture an opinion on their subspecific status. They are, however, small in size, as the average measurements show: wing, 356 mm., (348–371); culmen, 79.4 mm. (75–82.5); depth of bill, 24.6 mm. (23–26.5); tarsus, 87.4 mm. (86.5–89); outer toe, 110 mm. (108–113).

Gavia adamsi (Gray). YELLOW-BILLED LOON.—*1938:* Two adults of this species were seen on a small lake near Fort Ross, Somerset Island. These were readily attracted to a waving handkerchief and the female was taken. An immature loon of large size, presumably of this species, was observed on the sea at the east end of Bellot Strait on the same day.

Learmonth secured this species on the southeast coast of Somerset Island on June 30, 1938.

These records, coupled with one from the Melville Peninsula and reports from Foxe Basin (8), form a considerable north-eastward extension in the known range of *G. adamsi* and indicate that Ross's early record (3) for the Boothia Peninsula was within the normal range of the species.

Gavia arctica (Linnaeus). ARCTIC LOON.—*1938:* Five Arctic loons were seen near the post at Coral Harbour, Southampton Island, and an adult female was collected. Single birds were seen at Chesterfield, N.W.T., Arctic Bay, and Pond Inlet, Baffin Island. *1939:* One was observed at Churchill and one at Chesterfield.

Bailey collected two specimens, one at Lake Harbour at the end of May, 1932, and the other on Saddleback Island on June 17, 1930. He also secured an egg from a nest which was situated on an island off Big Island on June 18, 1930. This nest was located in the midst of a colony of common eider duck.

Gavia stellata (Pontoppidan). RED-THROATED LOON.—*1938:* This loon was observed at more posts and in greater numbers than any of the preceding. These observations were: at Chesterfield, N.W.T., two adults; Cape Dorset, Baffin Island, one adult; Fort Ross, Somerset Island, two adults; River Clyde, Baffin Island, one adult, two young; Pond Inlet, Baffin Island, two adults, two young; Churchill, Man., one adult; Coral Harbour, Southamp-

FIG. 1.

ton Island, one adult. A male was collected at Pond Inlet. It was still in full summer plumage. *1939:* The most common loon seen on the trip. One seen at Lake Harbour, Baffin Island; about 20 at Southampton Island; two at Port Harrison, Que.; one at Chesterfield, N.W.T.; one in Kennedy Harbour, Fort Ross, Somerset Island; and one at Pond Inlet, Baffin Island.

Bailey sent four breeding specimens from Lake Harbour. These had been taken in July, 1931, and in June 1932.

Learmonth collected five specimens on Somerset Island. These were breeding adults taken at Fort Ross and Port Leopold during June and July, 1938, June, 1939, and July, 1940.

Podilymbus podiceps (Linnaeus). PIED-BILLED GREBE.—Bailey secured a specimen which had been taken by a native in November, 1932, at Fair Ness, Baffin Island. This record has been published by Snyder (4).

Puffinus gravis (O'Reilly). GREATER SHEARWATER.—*1938:* Seen in some numbers off the Labrador coast near Makkovik on July 14. *1939:* First seen on July 11 in the western part of Belle Isle Strait. Several seen off Cartwright, Labrador, on July 12. On the return trip they were first met off Hamilton Inlet, Labrador, on September 20.

Fulmarus glacialis (Linnaeus). NORTHERN FULMAR.—*1938:* The most common bird at sea. Observed north to Lancaster Sound and adjacent latitude in Baffin Bay. *1939:* First encountered in the vicinity of Cape Chidley, where they were common, and in Gray Straits on July 19. None seen westward in Hudson Strait or in Hudson Bay. Abundant around Port Burwell and the Button Islands on August 16. Common in Davis Strait, Baffin Bay, Jones Sound, and even in Craig Harbour, Ellesmere Island. Fairly common in Lancaster Sound, Prince Regent Inlet, and at Fort Ross and the eastern end of Bellot Strait. Some were present in Admiralty Inlet and Adams Sound near Arctic Bay. A few in Pond Inlet and Cumberland Sound. Common all along the Labrador coast at least as far south as Cartwright. This was the most common bird at sea in the inlets and sounds north of Hudson Strait.

Learmonth secured a pair in July, 1939, and eight on May 4, 1940, at Port Leopold, Somerset Island, all in the light phase.

Cygnus columbianus (Ord). WHISTLING SWAN.—*1939:* Three observed flying over the shoreline of Southampton Island.

Marsh collected a male on May 30, 1937, at Eskimo Point.

Branta canadensis (Linnaeus). CANADA GOOSE.—*1938:* Seen only at Lake Harbour, Baffin Island, where two immatures were observed and one of them was collected on August 17. This specimen is referable to *B. canadensis canadensis*. *1939:* At Lake Harbour on August 15, fresh tracks and droppings were found on the sandy beach of a lake a few miles inland from the post.

Bailey found a nest containing four eggs at Ashe Inlet, Big Island, on June 17, 1930. He collected and preserved one egg.

A female was taken by Marsh on May 20, 1937, at Eskimo Point. This specimen appears to be of the form *leucopareia* (Brandt) as defined by Taverner (7). Its measurements are: culmen, 43 mm.; depth of bill, 19 mm.; tarsus, 69 mm.; middle toe and claw, 71 mm.; wing, 385 mm. The white cheek patches are divided by a broad black line along the throat and chin.

Branta bernicla Linnaeus. COMMON BRANT.—*1938:* A flock of about 10 birds of this species was observed in Lancaster Sound off Devon Island on August 27. *1939:* A flock of about 15 birds, seen flying eastward in Lancaster Sound on August 26, was probably of this species but was too far away for positive identification.

Learmonth secured a male at Port Leopold on June 21, 1939. He states that this species is "quite rare" on Somerset Island.

Chen hyperborea (Pallas). SNOW GOOSE.—*1939:* On September 6, at Pond Inlet, near the mouth of the Salmon River, numerous signs of this species were seen although the birds were reported to have been observed last on August 31.

Learmonth secured a female and a downy young on July 18, 1938, on the southeast coast of Somerset Island. The adult bird, on the basis of size, is referable to *C. hyperborea atlantica* Kennard, the greater snow goose. Its measurements agree well with those given by Kennard (2) for *atlantica* and are: wing, 459 mm.; exposed culmen, 64 mm.; depth of culmen, 36 mm.; middle toe and claw, 84 mm.; tarsus, 89 mm. In addition the bird has the heavy, robust build mentioned by Kennard. It is doubtful, however, if this bird is representative of the Somerset Island snow goose population.

Anas platyrhynchos Linnaeus. MALLARD DUCK.—*1938:* Several small flocks observed at Churchill, Man., on August 6. A flock of four, flying northward, was observed in Hudson Bay about 100 miles south of Chesterfield, probably off Tavani.

Anas rubripes Brewster. BLACK DUCK.—*1938:* Noted only at Churchill, where five birds were seen. *1939:* One seen on a small pond in the townsite at Churchill on August 7.

Dafila acuta (Linnaeus). PINTAIL.—*1938:* A large flock seen at Churchill over the marsh along the river and two birds observed in flight northward about 50 miles south of Chesterfield, N.W.T., on August 9. *1939:* Six observed in a small pond in the townsite at Churchill. Reported to be nesting around Chesterfield, but none seen during our stay.

A male was taken by Marsh at Eskimo Point on June 30, 1938.

Nettion carolinense (Gmelin). GREEN-WINGED TEAL.—*1938:* Two green-winged teal noted on August 6 at Churchill. *1939:* Two seen in the townsite slough at Churchill, August 7.

Clangula hyemalis (Linnaeus). OLD-SQUAW.—*1938*: The most frequently observed duck throughout most of the trip, especially in the waters about the Arctic Islands. Flocks of 20 to 100 were seen at Pond Inlet; River Clyde, Baffin Island; and Fort Ross, Somerset Island. A nest with eight eggs was found near the post at Coral Harbour, Southampton Island. Two downy young were collected from a flock of eight with parent at Chesterfield, N.W.T. *1939:* At Southampton Island two adults were seen with about 20 young which had not attained the power of flight. Two adults and about 20 young were also seen at Churchill. Several adults and about 40 young at Chesterfield. An adult was seen in an arm of Hazard Bay at Fort Ross. A raft of about 70 was found at the mouth of the Salmon River at Pond Inlet; three of these were young and still unable to fly.

Learmonth secured two adults at Port Leopold on June 20, 1940.

Histrionicus histrionicus (Linnaeus). HARLEQUIN DUCK.—*1939:* A pair was seen flying over the harbour at Lake Harbour, on July 22.

Somateria mollissima Linnaeus. COMMON EIDER DUCK.—*1938:* Next to the old-squaw, this was the most conspicuous duck. Three flocks of about 50 each were seen at River Clyde, Baffin Island. Individuals or small flocks were seen at Pond Inlet, Arctic Bay, Southampton Island, and Chesterfield. An adult female was taken from a flock of 12 at Chesterfield. This specimen is characteristic of the recently described race, *sedentaria* Snyder (5). A few small flocks were noted in Davis Strait. *1939:* This species was seen as follows: eleven flying in Gray Straits on July 19; 10 flying over Lake Harbour on July 22; several males seen at Sugluk West on July 25; one at Cape Dorset; a flock of 20 near Cape Wolstenholme on July 28; and about 20 near Southampton Island.

Bailey collected breeding birds at Lake Harbour, Saddleback Island, and on an island off Crooks Inlet, southern Baffin Island. He also secured eggs from a colony on Big Island which, according to his report, contained "hundreds of nests".

Learmonth took male specimens at Cresswell Bay on June 21, 1938, and at Port Leopold on July 12, 1939, and June, 1940. These birds are referable to *borealis* Brehm and probably represent the most westerly occurrence of this form.

Marsh collected a female at Eskimo Point on July 5, 1937.

Somateria spectabilis (Linnaeus). KING EIDER.—*1938:* This species was noted only sparingly. Three were noted off Cape Dorset, two were seen at Pangnirtung; a male was collected at Craig Harbour, Ellesmere Island. *1939:* Positively identified only at Southampton Island, where two were seen.

Bailey took one on July 11, 1932, at Lake Harbour.

Learmonth collected females at Cresswell Bay on June 15 and July 1, 1938, and a series of both sexes at Port Leopold in 1940. The earliest date among these is May 11.

Oidemia americana (Swainson). AMERICAN SCOTER.—*1938:* A pair of scoters, the male of which was all black, was seen flying southeast in Hudson Bay off Port Harrison on August 2.

Mergus serrator Linnaeus. RED-BREASTED MERGANSER.—Bailey secured an adult male of this species at Lake Harbour, Baffin Island, in 1932. The bird, which shows a few traces of the eclipse plumage, was presumably taken by natives in the fall.

Buteo lagopus (Brünnich). COMMON ROUGH-LEGGED HAWK.—*1938:* Pairs were noted at Hebron, Labrador; Lake Harbour, Baffin Island; Cape Smith, N.W.T.; Wakeham Bay and Cape Wolstenholme, Que. They apparently were nesting on the cliffs at each of these localities. Adult specimens were secured at Cape Smith on July 30, and at Cape Wolstenholme, August 14. *1939:* One observed a short distance inland from Port Harrison, probably nesting nearby.

Circus hudsonius (Linnaeus). MARSH HAWK.—*1938:* An adult male was seen on August 6 at Churchill, Man. *1939:* One in brown plumage was seen several miles inland from Churchill, August 7.

Falco rusticolus Linnaeus. GYRFALCON.—*1938:* Definitely identified only at Cape York, Greenland, where a white individual was observed flying over the sea near the coast. A freshly shed unsoiled feather which was picked up at Pangnirtung, Baffin Island, has been positively identified as white gyrfalcon. Dark falcons were seen at long range near Thule, Greenland, and near Hebron, Labrador. These were not identified, but the former was most likely a gyrfalcon. *1939:* A black adult was seen soaring over a cliff several miles inland from Lake Harbour on August 15.

Bailey took one at Lake Harbour on August 11, 1930.

Learmonth collected one at Bellot Strait on November 5, 1938.

Both of these birds are typically white specimens of *F. candicans* Gmelin.

Falco peregrinus Tunstall. PEREGRINE FALCON.—*1938:* Individuals were seen at Sugluk West, Que., and at Churchill, Man. A falcon observed at River Clyde, Baffin Island, was also believed to be *Falco peregrinus*. A family of four birds was found frequenting a

high precipitous cliff at Fort Ross, Somerset Island, and the adult male was collected. *1939:* One seen chasing two ravens at Arctic Bay. On September 20, while the *Nascopie* was off the Labrador coast near Hamilton Inlet, an immature bird spent four or five hours on the ship, apparently tired out.

The Fort Ross and Arctic Bay records appear to be the most northerly for this species in Canada.

Lagopus lagopus (Linnaeus). WILLOW PTARMIGAN.—*1939:* Three were seen several miles inland from Churchill on August 7.

Marsh collected two at Eskimo Point on May 3, 1937.

Lagopus rupestris (Gmelin). ROCK PTARMIGAN.—*1938:* This species was not common. Three females, one with three young, the others with one each, were observed at Sugluk West, Que. An adult male was seen at Pond Inlet and a female with one young was noted at Arctic Bay. Adult and juvenile specimens were collected at Sugluk and Arctic Bay.

Bailey took two on May 12, 1930, at Lake Harbour and a female on an island off Markham Bay on July 3, 1930.

Learmonth collected a juvenile male at Fort Ross on August 25, 1940.

Fulica americana Gmelin. AMERICAN COOT.—Learmonth preserved a specimen which was found dead on sea ice at the east end of Bellot Strait on October 30, 1937. This must be considered an accidental occurrence as the bird was far north of the normal range for the species.

Charadrius hiaticula Linnaeus. GREATER RINGED PLOVER.—*1938:* Found only on a gravelly bar at the mouth of the Salmon River, Pond Inlet, Baffin Island. Three immatures, of which two were collected, were observed. *1939:* Individuals believed to be of this species were seen at River Clyde.

Charadrius semipalmatus Bonaparte. SEMIPALMATED PLOVER.—*1938:* Plentiful throughout the region of Hudson Straits and north to Pangnirtung. Five adult and five juvenile specimens were taken, representing the following localities: Lake Harbour, Wakeham Bay, Sugluk West, Port Harrison, and Pangnirtung. *1939:* A common species, noted at the following posts: about 15 at Cape Dorset, four at Southampton Island, one at Port Harson, common at Churchill, 10 at Chesterfield, and one at Cape Wolstenholme. An individual which may have been of this or the preceding species was seen at Fort Ross.

Learmonth's specimen of this species, taken on August 26, 1940, at Fort Ross, is of interest in view of the occurrence of the greater ringed plover in similar latitudes on adjacent Baffin Island. It also appears to represent a northward extension in the known range of the semipalmated plover. The specimen is a juvenile male.

Marsh secured a male at Eskimo Point on July 5, 1937.

Pluvialis dominica (Müller). GOLDEN PLOVER.—*1939:* Eight seen at close range on Southampton Island.

Squatarola squatarola (Linnaeus). BLACK-BELLIED PLOVER.—Learmonth took a pair at Cresswell Bay on June 16, 1938.

Arenaria interpres (Linnaeus). COMMON TURNSTONE.—*1938:* An adult and two downy young were collected at Coral Harbour, Southampton Island. Many small flocks were observed at Craig Harbour. Four juvenile specimens were collected. *1939:* Six, of which two were young that were unable to fly, were seen at Southampton Island. Several observed at Churchill.

A pair was collected at Cresswell Bay by Learmonth on June 17, 1938.

Phaeopus hudsonicus (Latham). HUDSONIAN CURLEW.—*1938:* Seen only at Churchill, Man. Several adult birds observed there on August 6 were highly agitated, probably concerned with their young concealed in the grass. One of the agitated birds was collected and proved to be a female. On the same date fairly large flocks of Hudsonian curlew were seen along the Churchill River. *1939:* About 30 were seen at Southampton Island, two at Churchill, and five at Chesterfield.

Totanus melanoleucus (Gmelin). GREATER YELLOWLEGS.—*1938:* About 50 birds of this species were observed at Churchill, in flocks of from 3 to 10. A female was collected on August 6.

Totanus flavipes (Gmelin). LESSER YELLOWLEGS.—*1938:* A small flock was observed on August 6 along the river near Churchill and several were seen in the townsite slough. *1939:* Several seen in small ponds near Churchill.

Calidris canutus Linnaeus. KNOT.—Learmonth collected a female on June 14 and a male on June 16, 1938, at Cresswell Bay.

Arquatella maritima (Brünnich). EASTERN PURPLE SANDPIPER.—*1938:* A small flock seen at Pond Inlet. There were many immatures at River Clyde. Three males and two females were collected at Clyde. *1939:* One seen at Fort Ross, Somerset Island.

Bailey collected two at Lake Harbour on June 1, 1932.

Learmonth secured a male on June 14 and a female on June 15, 1938, at Cresswell Bay.

Pisobia melanotos (Vieillot). PECTORAL SANDPIPER.—*1938:* An adult male was collected from a flock of 12 at Churchill.

Pisobia fuscicollis (Vieillot). WHITE-RUMPED SANDPIPER.—*1938:* The most commonly observed wader except the semipalmated sandpiper for the region as a whole during this year, even greatly exceeding the latter north of Hudson Straits. Three specimens were collected at Coral Harbour, Southampton Island, including a semidowny chick and a fully feathered juvenile. Juveniles were also taken at Chesterfield, N.W.T., River Clyde, and Arctic Bay. The latter was the most northerly observation. *1939:* Two were seen at Cape Dorset, five at Southampton Island, and two at Pond Inlet.

Learmonth secured two at Fort Ross on August 26, 1940.

Pisobia bairdi (Coues). BAIRD'S SANDPIPER.—*1938:* Several adults were seen and one collected at Chesterfield. Two juveniles, with down still adhering to the occiput were flushed from a small inland pool at Arctic Bay and one was collected.

Learmonth collected two specimens at Fort Ross on August 26 and September 11, 1940.

Pisobia minutilla (Vieillot). LEAST SANDPIPER.—*1938:* Seen only at Churchill about the townsite slough on August 6.

Pelidna alpina (Linnaeus). DUNLIN.—*1938:* This species was found in large numbers at Churchill and Chesterfield and specimens in the very handsome juvenile plumage were collected at both places. *1939:* Two seen at Chesterfield.

Limnodromus griseus (Gmelin). DOWITCHER.—*1938:* This species was migrating in fairly large numbers at Churchill and two juvenile specimens were taken. *1939:* One observed at Churchill near Fort Prince of Wales.

Micropalama himantopus (Bonaparte). STILT SANDPIPER.—*1938:* A flock of six juveniles was observed at Churchill. Two specimens collected were both females. *1939:* Several observed at Churchill.

Ereunetes pusillus (Linnaeus). SEMIPALMATED SANDPIPER.—*1938:* A very common shorebird on Southampton Island and on both coasts of Hudson Bay. A family of downy young was collected on Southampton Island and semidownies were taken at Port Harrison and Chesterfield. *1939:* This species and the semipalmated plover were the most widely distributed shorebirds observed during the trip of this year. Observed as follows: three at Southampton Island, one at Port Harrison, several at Churchill, common at Chesterfield, and about six at Fort Ross.

Crocethia alba (Pallas). SANDERLING.—*1938:* One seen at Pond Inlet. *1939:* Two seen on the sandy beach at Clyde River.

Learmonth secured a male on June 15 and a female on June 17, 1938, at Cresswell Bay. He also collected two at Fort Ross on August 26, 1940.

Phalaropus fulicarius (Linnaeus). RED PHALAROPE.—*1938:* Encountered in some numbers on Southampton Island, where two adult males and two partly feathered downy young were collected. Also several were seen at Chesterfield, where a juvenile was taken.

Bailey collected specimens at Lake Harbour on June 15, 1932, and a pair on Jubilee Island on June 29, 1930.

Learmonth secured specimens at Cresswell Bay about the middle of June, 1938.

Lobipes lobatus (Linnaeus). NORTHERN PHALAROPE.—*1938:* Five seen at Churchill. *1939:* Very common at Churchill. A flock of six seen at sea near Lake Harbour on August 14 were believed to be this species.

Stercorarius parasiticus (Linnaeus). PARASITIC JAEGER.—*1938:* A flock of eight seen at Southampton Island. Also noted in some numbers at Churchill, Chesterfield, and Craig Harbour and at Thule, Greenland. Two adults were collected. *1939:* Two were seen at Southampton Island and one at Churchill. Two observed at Chesterfield were chasing a snowy owl.

Learmonth collected two light-phased birds on June 17, 1938, at Cresswell Bay and a dark female on June 8, 1940, at Port Leopold.

Stercorarius longicaudus Vieillot. LONG-TAILED JAEGER.—*1938:* Two adults seen at Cape Dorset and two at Coral Harbour, Southampton Island. An adult was collected at Fort Ross, Somerset Island. *1939:* On July 19 at Cape Chidley and in Gray Strait there were at least one hundred and fifty. One was seen at Southampton Island; one at sea,

south of Southampton Island, on August 12; six seen near a large iceberg in Davis Straits off Frobisher Bay on August 17; one in Prince Regent Inlet on August 27; and one at Pond Inlet.

Learmonth collected males of this species, one on June 18, 1938, at Cresswell Bay and one in July, 1939, at Port Leopold.

Larus hyperboreus Gunnerus. GLAUCOUS GULL.—*1938:* The commonest gull in the area covered. It was observed at all ports of call except Churchill, Chesterfield, and River Clyde. A breeding colony of about 20 pairs was observed on the high, inaccessible cliffs near Glasgow Falls, Lake Harbour, on July 20. A male was collected at Craig Harbour. This bird possessed a vague, pinkish blush on the underparts, strongest on the breast. *1939:* The commonest and most widespread gull of the trip. Observed at nearly every port as follows: a few at Hebron, some at Cape Chidley; a few at Lake Harbour, Wakeham Bay, Sugluk West, Cape Dorset, Wolstenholme, Southampton Island, and Port Harrison; observed occasionally in Davis Straits and Baffin Bay; a few at Craig Harbour; adults and immatures at Pond Inlet and Pangnirtung; observed at Hebron, September 18.

Bailey collected a male on June 1, 1931, at Lake Harbour and a female off Big Island on June 18, 1930. He also collected eggs almost ready to hatch on an island off Big Island on June 18, 1930.

Learmonth collected a specimen at Port Leopold in July, 1939, and two at the same place on June 8, 1940.

Larus marinus Linnaeus. GREAT BLACK-BACKED GULL.—*1938:* The only northern observation of this species was that of an individual at Hebron, Labrador, in September. *1939:* Not seen north of Belle Isle Strait, where they were rather common.

Larus argentatus Brünnich. HERRING GULL.—*1938:* Fairly common. All those in Hudson Bay and Hudson Strait that could be scrutinized closely seemed to have the dark wing tips of *L. argentatus smithsonianus.* Specimens in fully adult plumage collected at Fort Ross and Arctic Bay were typical of the northern form, *L. argentatus thayeri* Brooks. These two birds possessed light *brown* irides and *purplish-pink* eyelids. *1939:* A few seen at Lake Harbour on July 22; a few at Southampton and many at Churchill and Chesterfield, these all being of the race *L. argentatus smithsonianus* Coues. *L. argentatus thayeri* was observed as follows: two were seen in ice pack in Prince Regent Inlet on August 29; many, of which 8 or 10 were young and unable to fly, at Fort Ross; about 15, of which a few were young birds, at Arctic Bay; and four at Pond Inlet.

Learmonth collected several specimens of the northern form, *L. argentatus thayeri*, including breeding adults and juveniles at Port Leopold and Fort Ross.

Larus kumlieni Brewster. KUMLIEN'S GULL.—*1938:* Gulls which were almost certainly of this species were seen near Cape Wolstenholme on July 29 and on August 14. Information to the effect that these birds nested near Erik Cove was secured locally. *1939:* A few were seen in the harbour at Wolstenholme on July 28 and August 13.

Larus philadelphia (Ord.). BONAPARTE'S GULL.—*1938:* A few seen at Churchill. *1939:* Common in the harbour at Churchill.

Pagophila alba Gunnerus. IVORY GULL.—*1938:* A few were seen in Lancaster Sound and Prince Regent Inlet and one was observed with glaucous gulls at Craig Harbour. *1939:* Two seen at Craig Harbour and two in Prince Regent Inlet on August 27.

Learmonth reports this species as quite abundant in spring but very scarce in summer on south Somerset Island. He collected two females on July 6, 1938, and two on June 22, 1940, at Port Leopold.

Rissa tridactyla (Linnaeus). KITTIWAKE.—*1938:* A fairly common species. Observed along the Labrador and Davis Strait as far north as Devon Island. *1939:* Observed commonly. A few in Belle Isle Strait, off the Labrador coast and around Cape Chidley; (not seen in Hudson Strait or Bay); a few in Davis Strait and Baffin Bay; several at Craig Harbour; a few in Lancaster Sound and Prince Regent Inlet; two at Pond Inlet; several at Clyde Inlet; a few in Cumberland Sound; common off the Labrador coast on the return trip, especially off Hamilton Inlet.

Learmonth collected this species at Bellot Strait on July 6, 1938, and at Port Leopold on June 23, 1939, and June 10, 1940.

Xema sabini (Sabine). SABINE'S GULL.—*1939:* Three seen at Southampton Island and one off Clyde Inlet on September 7.

Bailey secured a specimen during the last week of July, 1932, on Big Island.

Sterna paradisaea Brünnich. ARCTIC TERN.—*1938:* Common at Southampton Island and at Churchill; a few seen at Chesterfield; not noted elsewhere. *1939:* Abundant at Southampton Island; common in Churchill harbour; about eight were seen at Chesterfield.

Two taken at Cresswell Bay by Learmonth on June 18 and 23, 1938; also a specimen collected at Bellot Strait on June 23, 1939.

Alca torda Linnaeus. RAZOR-BILLED AUK.—*1938:* Last seen off the Labrador coast near Cartwright.

Uria aalge (Pontoppidan). COMMON MURRE.—*1938:* Not identified north of Belle Isle Strait. *1939:* Common in Belle Isle Strait and a few off the lower Labrador near Cartwright.

Uria lomvia (Linnaeus). THICK-BILLED MURRE.—*1938:* Common. Observed in Hudson Strait almost to the western entrance but not seen in Hudson Bay. Observed in Prince Regent Inlet, Admiralty Inlet, Lancaster Sound, and Baffin Bay north to Thule, Greenland. *1939:* Common at Cape Chidley and throughout Hudson Strait (but none in Hudson Bay) and northward in Davis Strait and Baffin Bay; a few seen at Craig Harbour; many in Prince Regent Inlet; a few in Lancaster Sound, near Pond Inlet; and at Clyde River. Murres unable to fly were seen in Prince Regent on August 27. Some of these were young, others may have been adults in the eclipse moult.

Bailey collected two specimens at Lake Harbour.

Three examples were taken by Learmonth in June and July, 1938 and 1939, on the southeast coast of Somerset Island, and five at Port Leopold on June 10, 1940.

Alle alle (Linnaeus). DOVEKIE.—*1938:* Abundant in Davis Strait and Baffin Bay during the latter part of August. Observed as far west as Lancaster Sound, about 87° west longitude. *1939:* First seen in Baffin Bay, east of Devon Island, on August 21; common in Prince Regent Inlet on August 31; some seen in Lancaster Sound and Admiralty Inlet. Flocks of 20 to 40 seen in Davis Strait near Clyde River but none south of Clyde.

Cepphus grylle (Linnaeus). BLACK GUILLEMOT.—*1938:* Distributed about as the last, but the most northerly observation was off Devon Island in Davis Strait. A female was collected at Pond Inlet. *1939:* Rather common and widespread, having been seen as follows: a few near Hebron; a few at Cape Chidley; one at Cape Smith; several at Port Burwell on August 16; one at Craig Harbour; a few in Lancaster Sound on August 26; several in Prince Regent Inlet on August 27. It was reported to winter at the edge of the ice-floe in Admiralty Inlet and Pond Inlet.

Bailey found this bird common about Lake Harbour and collected six breeding specimens. He secured an egg from a hollow in rough gravel far under a thin shelf of rock on July 3, 1930, on an island 25 miles west of Crooks Inlet.

Learmonth collected specimens at Port Leopold on June 15 and 27, 1939, July 6, 1938, and in May and June, 1940.

Fratercula arctica (Linnaeus). ATLANTIC PUFFIN.—*1938:* The most northern observation was off Rigolet, Labrador. *1939:* Some in Belle Isle Strait and one seen off Cartwright, Labrador, on July 12.

Nyctea nyctea (Linnaeus). SNOWY OWL.—*1938:* One adult was seen near Pond Inlet, Baffin Island. *1939:* One seen at Chesterfield and one immature specimen, on an ice-floe in Prince Regent Inlet on August 27.

Bailey took one at Lake Harbour on September 29, 1930, and also collected a young male from a nest at Lake Harbour in June, 1930.

Marsh collected specimens on February 28, May 20, and June 1, 1936, at Eskimo Point.

Asio flammeus (Pontoppidan). SHORT-EARED OWL.—*1939:* One seen several miles inland from Churchill.

Marsh collected a specimen on September 1, 1937, at Eskimo Point. He comments that the species is not common there.

Otocoris alpestris (Linnaeus). HORNED LARK.—*1938:* Observed north to Fort Ross and Arctic Bay, at which points it was as common as farther south. Seventeen specimens were taken as follows: two at Port Burwell, four at Lake Harbour, three at Sugluk West, four at Port Harrison, two at Arctic Bay, and two at Fort Ross. *1939:* One at Port Harrison; common at Churchill; one immature on ice-floe near ship in Prince Regent Inlet on August 28; about eight at Fort Ross and 10 at Arctic Bay.

Bailey took a specimen on May 22, 1930, at Lake Harbour.

Learmonth collected specimens at Cresswell Bay on June 16, 1938, and at Fort Ross on August 26, 1940.

Iridoprocne bicolor (Vieillot). TREE SWALLOW.—*1938:* A single bird was seen near the post at Chesterfield on August 10. It flew off, high over the water, in a southerly direction and could not be collected. *1939:* Four seen around small ponds in the townsite at Churchill on August 7.

Corvus corax Linnaeus. NORTHERN RAVEN.—*1938:* Not common, but a few seen at Hebron and Wakeham Bay and many at Pond Inlet. Individuals were seen at Lake Harbour, Craig Harbour, Port Burwell, and Cape Dorset. One was collected at Pond Inlet. *1939:* One at Lake Harbour, July 23; six at Cape Smith; one at Craig Harbour; two at Arctic Bay; three at Pond Inlet; about six at Pangnirtung; and one at Hebron, on September 18.

Sialia currucoides (Bechstein). MOUNTAIN BLUEBIRD.—*1938:* A male was collected from a small clump of spruces a few miles south of Churchill on August 6. This is the first definite record of the species at Churchill, but suggests the identity of a young bluebird taken by Twomey (9) on June 10, 1931, but lost before it could be identified.

Oenanthe oenanthe (Linnaeus). WHEATEAR.—*1938:* A family group was observed about half a mile from Cape Wolstenholme, Que., on August 14, and a specimen in the scale-marked juvenile plumage was taken. Immatures were observed at Arctic Bay and Pangnirtung, Baffin Island. Two were collected at Arctic Bay and one at the latter post. Constable Fitzrandolph, who was stationed at Lake Harbour, showed me on August 17 where a pair had nested in a rock-faced terrace near the Royal Canadian Mounted Police post earlier in the summer. The frequency with which this species was encountered in 1938 during the hurried *Nascopie* cruise would indicate that an increase in the number of wheatears has taken place in Baffin Island in the last 10 years (1). Soper writes (6) that this species was ". . . . . one of the rarest birds of Baffin Island."

Anthus spinoletta (Linnaeus). AMERICAN PIPIT.—*1938:* The most abundant land bird of the high rocky coastal region about Hudson Strait. Eleven specimens were taken, including juveniles at Cape Dorset, Lake Harbour, and Port Harrison. Other localities where specimens were taken were Port Burwell and Cape Wolstenholme. *1939:* Found rather commonly at most ports of call. Common at Hebron, Lake Harbour, Sugluk, Harrison, and Churchill. Several at Port Burwell, Wakeham Bay, Cape Dorset, Wolstenholme, Cape Smith, and Chesterfield. Four were definitely identified at Fort Ross; two at Arctic Bay, four at Pond Inlet, and about four at Pangnirtung.

Dendroica virens (Gmelin). BLACK-THROATED GREEN WARBLER.—*1938:* A male came aboard the *Nascopie* while the ship was out of sight of the coast of Labrador, about latitude 53° North, on September 17. The bird was in an exhausted condition, and though given food and water, died the next day.

Acanthis hornemanni (Holboell). ARCTIC REDPOLL.—*1938:* Observed only at Churchill, where an adult male and a juvenile were taken.

Acanthis linaria (Linnaeus). REDPOLL.—*1938:* Observed only at Sugluk West, Hebron, and Pangnirtung. A newly made nest was found at Hebron on July 15. Two adult females and two juveniles collected at Sugluk are typical *linaria*, while a female taken at Pangnirtung is *rostrata*. It is decidedly buffy in colour, indicating immaturity, and has a wing 80 mm. in length.

Passerculus sandwichensis (Gmelin). SAVANNAH SPARROW.—*1938:* Two adult specimens were collected at Wakeham Bay. The only other locality where this species was noted was Churchill, where it was common and two specimens were collected. *1939:* A few seen at Sugluk, two at Port Harrison, and several at Churchill.

Spizella arborea (Wilson). TREE SPARROW.—*1938:* Met with only at Churchill, where three juveniles and one adult were collected on August 6.

Zonotrichia leucophrys (Forster). WHITE-CROWNED SPARROW.—*1938:* This species was common at Hebron, Labrador, inhabiting the scrub willows. On July 15 many juveniles just able to fly were observed and two were caught by hand. One singing male was seen at Port Burwell, N.W.T., but could not be secured. *1939:* At Hebron a few adults were seen on July 17 and a nest with five eggs was found. A few seen at Lake Harbour on July 23; at Churchill it was common and one was heard singing at Hebron on September 18.

Calcarius lapponicus (Linnaeus). LAPLAND LONGSPUR.—*1938:* This was an abundant species at nearly all ports, but was not observed at Hebron, Lake Harbour, Port Burwell, or Wakeham Bay. An adult male was collected at Craig Harbour, Ellesmere Island. It was the only one seen there and appears to represent a northern record for the species. Twenty-two specimens were taken, including juveniles on the breeding grounds at Cape Dorset, Fort Harrison, and Southampton Island. *1939:* Found to be abundant at Southampton Island; a few seen at Churchill; common at Chesterfield; one seen on the ice in Prince Regent Inlet, about 30 miles from Fort Ross, on August 28; six at Arctic Bay; two at Pond Inlet; four at Clyde River; a few at Pangnirtung; and an immature came aboard the ship on September 16 in Davis Strait.

Bailey secured specimens on May 14 and 16, 1930, at Lake Harbour.

Marsh took a female at Eskimo Point on July 5, 1936.

Learmonth got this species on June 18, 1938, at Cresswell Bay and on June 21 at Bellot Strait. A common bird on Somerset Island.

Calcarius pictus (Swainson). SMITH'S LONGSPUR.—*1938:* An adult female and a juvenile collected at Churchill on August 6. *1939:* Several birds observed in company with Lapland longspurs about the gravel flats in the townsite of Churchill.

Plectrophenax nivalis (Linnaeus). SNOW BUNTING.—*1938:* The most abundant species of the trip. It was noted at every port with the exception of Hebron. Seventeen specimens, representing a variety of plumages, were taken. *1939:* The most commonly observed land bird, being seen at every port except Hebron and Wakeham Bay.

Bailey collected specimens at Lake Harbour on May 22, 1930. He also collected two sets of eggs at Lake Harbour on July 2 and 23, 1930.

Learmonth found the snow bunting common on Somerset Island and took examples on June 20, 1938, at Cresswell Bay and on September 10 and 11, 1940, at Fort Ross.

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EFFECTS OF PHENOTHIAZINE ON HORSES

I. STUDIES IN HAEMATOLOGY AND PHARMACOLOGY[1]

By W. E. Swales[2], H. B. Collier[3], and Della Allen[4]

Abstract

Studies have been made on the anaemia and cythaemolytic icterus produced by 60 and 70 gm. doses of phenothiazine to horses weighing approximately 1200 lb. The continued and prolonged haemolytic effect of a single dose may be serious in a horse having a large proportion of microcytic erythrocytes. No evidence of damage to the liver was obtained, but clinical observations confirm a previous report that severe injury to the urinary system may result from a dose of 60 gm. of phenothiazine to a 1200 lb. horse.

A dose of 30 gm. removed all the strongyloid nematodes and did not cause haemolysis. Less than half the dose could be recovered from the excreta of treated animals. The effects of phenothiazine on horses are similar to those of sulphanilamide and sulphapyridine in susceptible humans. The possibility of over-stimulation of the spleen with the consequent hyperactivity causing prolonged haemolysis is mentioned.

The development of phenothiazine as an anthelmintic has led to trials with all domestic animal species. The high efficiency of this chemical against the strongyloid nematodes in the caecum and large intestine of horses was determined by Harwood, Habermann, Roberts, and Hunt (8) and later by Grahame, Morgan, and Sloane (6), Knowles and Franklin (10), and Errington and Westerfield (3). Although all these studies confirmed the efficiency of phenothiazine, there was no general agreement regarding its toxicity for horses. The descriptions of toxic symptoms were at first confined to the mention of bronzed mucous membranes, slight malaise, abdominal pain, and anaemia. More detailed studies were later described by Errington (2).

The work described in this paper has been confined to observations on two horses, but a great many data on haematological and chemical findings have been obtained and are deemed worthy of publication at this time.

The haemolytic anaemia that follows administration of relatively large doses of phenothiazine to horses is of considerable interest, particularly because of its relationship to the anaemias sometimes brought about by sulphonamide therapy (5). An anaemia following the oral administration of phenothiazine to man, rats, and rabbits was first recorded by Thomas *et al.* (15). It is probable that the equine anaemia described herein was similar to that in other mammals.

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Experiment I

Subject

Horse *A*, six years old, gelding; good health and condition; weight 1250 lb.

Procedure

Normal levels of haemoglobin were obtained on five occasions over a period of 28 days by the Dare haemoglobinometer. Erythrocyte, leucocyte, and strongyloid nematode counts were made by standard methods. Seventy grams of pure phenothiazine were administered in the feed, after which all faeces and urine were collected separately for 80 hr. Blood samples, commencing at two hours after dosing, were collected at intervals for 34 days; from these samples the bilirubinaemia was measured by the indirect Van den Bergh method as modified by Malloy and Evelyn (11) to give more complete recovery of pigment; the haematological measurements were made as before.

The results are presented in Table I. This preliminary experiment served to show, and measure the intensity of, the anaemia and apparent cythaemolytic icterus produced in this animal. The anthelmintic effect was completely efficient; worms were evacuated in the faeces between the 24th and 48th hours as follows:

Hours after treatment	Worms passed (estimated)	
	Cylicostomes	Large Strongyles
24 – 26	37,200	31
26 – 29	9900	42
29 – 48	Very few	10

The quantitative tests for nematode eggs dropped from the average pre-treatment figure of 1460 eggs per gram to zero on the second day after treatment. All tests remained negative for 18 days and no quantitative test was positive until two months after treatment.

The clinical effects of the treatment were not severe. At the end of 24 hr., frequent and apparently painful urination occurred and there was a noticeable decrease in appetite. The normal 24 hr. faecal output of 48 lb. was reduced to 35 lb., but returned almost to normal on the second day. The mucous membranes of the eye and mouth were definitely bronzed 48 hr. after treatment and remained coloured for 16 days. The horse was not distressed by a run of about two miles on the fourth day following treatment.

The Recovery of Phenothiazine and Derivatives

Phenothiazine and its derivatives in the faeces, blood, and urine were estimated by the methods previously described (13), with certain modifications. Hydrogen peroxide was found to be unsuitable for oxidation of leucophenothiazone to the red pigment, and ferric chloride was substituted for it. One drop of 1% ferric chloride in 7*N* hydrochloric acid was added to the acid solution in the colorimeter tube and a reading taken. Then the pigment

TABLE I

HORSE *A*, EXPT. I

Changes in blood levels following a dose of 70 gm. of phenothiazine

	Haemoglobin		Erythrocytes		Bilirubin, mg., %
	Gm., %	Change, %	Millions/ mm.^{3}	Change, %	
Pretreatment normals	11.2 (σ = 0.1)		6.9 (σ = 0.5)		
Post-treatment values after:					
2 hr.	11.2	0	6.6	− 4	1.4
1 day	11.2	0	6.7	− 3	3.1
2 days	10.5	− 6	6.6	− 4	4.7
3 "	10.4	− 7	6.2	−10	3.7
4 "	10.5	− 6	5.5	−20	3.5
6 "	9.6	−14	5.8	−16	3.5
7 "	9.6	−14	5.7	−17	3.3
8 "	9.9	−12	5.7	−17	3.3
10 "	9.9	−12	5.7	−17	2.9
11 "	9.3	−17	5.8	−16	3.3
12 "	9.4	−16	5.4	−22	3.0
13 "	9.0	−20	5.0	−28	3.3
15 "	8.8	−21	4.5	−35	3.3
20 "	—	—	5.1	−26	1.9
24 "	9.8	−13	5.5	−20	1.6
27 "	10.3	− 8	6.3	− 9	1.3
34 "	11.0	− 2	6.9	0	0.9

was bleached by addition of a small crystal of stannous chloride and a second reading taken to represent the blank. The difference was calculated as phenothiazone.

This method appeared satisfactory for blood, except when highly jaundiced. In such cases, changes took place in pigments other than phenothiazone. When normal sheep urine was treated as above mentioned, no colour change was observed, but normal horse urine contained a substance that gave a purplish colour with ferric chloride and was reduced by stannous chloride. Unfortunately, since the concentration of this chromogen in normal horse urines was not constant, it was impossible to establish satisfactory blank values. An average blank has been deducted from the tabulated figures for urine, but the total must be regarded as only approximate, although the error is believed to be less than 10% of the total output of phenothiazone.

In the faeces, traces of oxidation products were found to be present. An aliquot sample of dried and ground faeces was extracted with boiling water under quantitative conditions, and a suitable volume of this extract was acidified and treated with ferric chloride and stannous chloride, as described above, in order to estimate the phenothiazone content. Normal faeces gave no significant reaction, under these conditions.

These analyses resulted in the recovery of the amounts shown in Table II. The discrepancy between the amounts recovered from the horse and the sheep (13) could not be explained; the possibility of a large percentage being retained in host tissues was considered.

TABLE II

HORSE *A*, EXPT. I

Detection and recovery of phenothiazine and oxidation derivatives

Blood serum

Post-treatment times, hr.	Total leucophenothiazone, %
5½	0.0060
24	0.0035
49	0.0010
77	0.0006

Faeces

Post-treatment periods, hr.	Dry weight, gm.	Phenothiazine		Oxidation products (as leucophenothiazone), gm.
		Gm.	Gm./hr.	
7 – 22	2593	7.57	0.50	2.80
22 – 23	334	1.12	2.24	0.45
23 – 28	512	1.24	0.24	0.52
28 – 31	490	1.23	0.61	0.36
31 – 47	2860	1.56	0.10	1.37
47 – 49	418	0.28	0.14	0.14
49 – 55	1038	0.35	0.06	0.52
		Total = 13.4		Total = 6.16 = 5.7 gm. phenothiazine

Urine

Post-treatment periods, hr.	Volume, ml.	Total leucophenothiazone		
		%	Gm.	Gm./hr.
0 – 1	1060	0.004	0.05	0.05
1 – 9	975	0.181	1.77	0.22
9 – 24	2000	0.268	5.35	0.36
24 – 33	1030	0.151	1.56	0.22
33 – 49	1100	0.099	1.09	0.07
49 – 58	1000	0.035	0.35	0.04
58 – 72	1100	0.043	0.48	0.03
72 – 80	1050	0.016	0.17	0.02
Washings	1230	0.058	0.71	—
			Total = 10.8 = 10.0 gm. phenothiazine	

NOTE: *Total recovery = 29.1 gm. phenothiazine (= 41.6% of dose).*

Experiment II

Subject

Horse *B*, five years old, gelding; good health and condition, weight approximately 1200 lb.

Procedure

Normal levels of haemoglobin, blood cells, blood bilirubin, erythrocyte volume, erythrocyte fragility, blood sedimentation rates, faecal output, and strongyloid nematode egg output were measured on several occasions over a period of two months. The accuracy of haemoglobin determinations was increased by the use of a calibrated acid haematin standard in a Klett-Summerson photoelectric colorimeter. The tests for erythrocyte fragility were made by using a series of sodium chloride solutions, 9.5 cc. of each, ranging from 0.85 to 0.50% in steps of 0.02; 0.5 cc. of blood was added to each tube and that percentage of sodium chloride that brought about a trace of haemolysis (2% as determined colorimetrically against a 100% distilled water check) was taken as the end point.

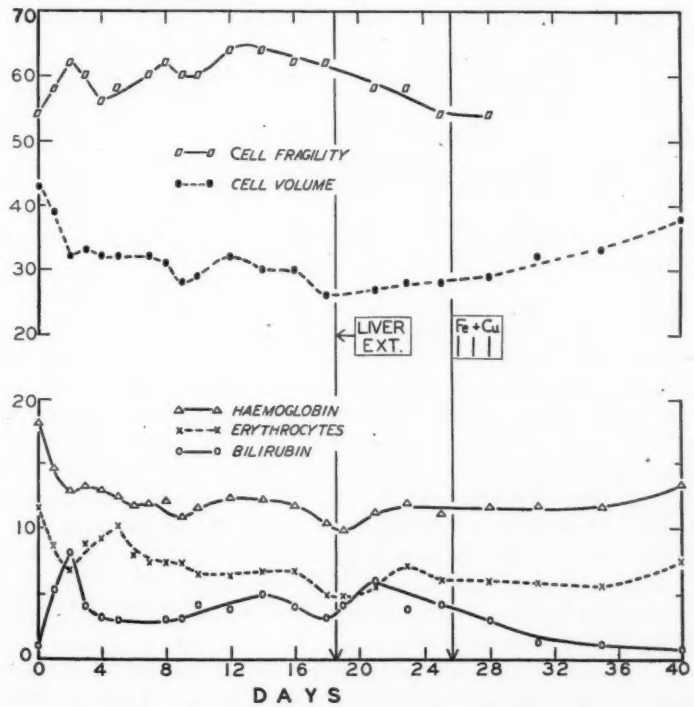

FIG. 1. *Blood values of Horse B for the period of 40 days following a dose of 60 gm. of phenothiazine. Cell fragility = % NaCl × 100; cell volume = %; haemoglobin = gm., %; erythrocytes = millions per cu. mm.; bilirubin = mg., %.*

The horse was dosed with 60 gm. of recrystallized phenothiazine in feed, which was readily consumed when a half pint of molasses was admixed. All the faeces were collected for five days and the urine for six days. Blood samples were taken at intervals for 40 days; the measurements made are shown in Table III and Figs. 1 and 2.

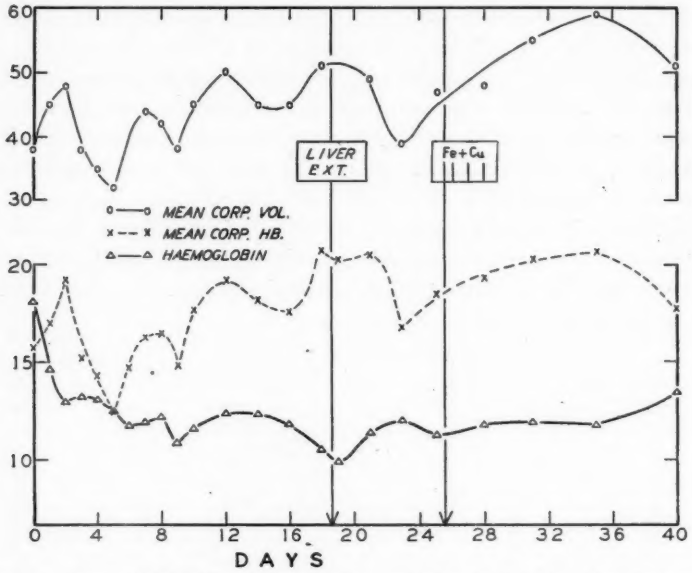

FIG. 2. *Further blood values of Horse B (same period as in Fig. 1). Mean corpuscular volume = μ^3; mean corpuscular haemoglobin = $\mu\mu$g.; haemoglobin = gm., %.*

In addition to the measurements shown in Table III observations were made on "vital stained" and Giemsa stained smears from each blood sample for the presence of nucleated erythrocytes and reticulocytes. The examinations for reticulocytes were made by the conventional "vital staining" method, using 1% cresyl blue in saline and also by the alcoholic cresyl blue staining of a wet smear (16). No true reticulocytes or nucleated erythrocytes were found during the experiment; however, on the seventh day after treatment a number of the erythrocytes had a small dark body in or just beneath the membrane. These bodies reached their maximum on the 12th day after treatment, when they were present in approximately 70% of the erythrocytes; after this they decreased until the 36th day, when none could be found. They were apparently nuclear fragments, and were comparable with the Howell-Jolly bodies of human erythrocytes*.

The faecal urobilinogen was determined by the method of Watson (16) in order to throw light upon the nature of the icterus. Table IV shows the

* *The recent finding of A. P. Richardson (Federation Proc. 1 : 164. 1942.), that Heinz bodies are formed during sulphanilamide anaemia, suggests that the bodies observed in the horse may be of a similar nature.*

TABLE III

HORSE *B*, EXPT. II

Blood changes following a dose of 60 gm. of phenothiazine

—	Erythrocytes		Haemoglobin		Mean corpuscular haemoglobin		Erythrocyte volume		Mean corpuscular volume		Cell fragility		Sedimentation rate				Bilirubin		Leucocytes	
													1st hour		2nd hour					
	Millions per mm.³	Change, %	Gm., %	Change, %	μμg.	Change, %	%	Change, %	μ³	Change, %	NaCl, %	Change, %	%	Change, %	%	Change, %	Mg, %	Change, %	Thousands per mm.³	Change, %
Pretreatment	11.5 (σ=0.3)		18.1 (σ=0.5)		15.8 (σ=0.5)		43 (σ=2)		38 (σ=2)		0.54 (σ=0.00)		37 (σ=9)		46 (σ=7)		1.1 (σ=0.2)		9.1	
Post-treatment																				
5 hr.	11.8	3	18.0	− 1	15.3	− 3	44	2	37	− 3	0.56	4	16	−57	23	−50	1.2	9	9.2	1
1 day	8.6	−25	14.6	−19	17.0	8	39	− 9	45	18	0.58	7	12	−68	27	−41	5.2	373	10.9	20
2 days	6.7	−43	12.9	−29	19.2	22	32	−26	48	26	0.62	15	—	—	—	—	8.0	628	16.8	85
3 days	8.7	−24	13.2	−27	15.2	− 4	33	−23	38	0	0.60	11	48	30	58	26	4.2	282	14.4	58
4 days	9.1	−21	13.0	−28	14.3	−10	32	−26	35	− 8	0.56	4	55	49	60	31	3.3	200	12.4	36
5 days	10.1	−12	12.4	−31	12.3	−22	32	−26	32	−16	0.58	7	57	54	61	33	3.1	182	8.3	− 9
6 days	7.9	−31	11.6	−36	14.7	− 7	—	—	—	—	—	—	—	—	—	—	—	—	8.6	− 6
7 days	7.3	−37	11.8	−35	16.2	3	32	−26	44	16	0.60	11	55	49	60	31	3.1	182	9.5	4
8 days	7.3	−37	12.0	−34	16.4	4	31	−28	42	11	0.62	15	53	43	59	28	3.1	182	10.7	18
9 days	7.3	−37	10.8	−40	14.8	− 6	28	−35	38	0	0.60	11	60	62	64	39	3.2	191	11.8	30
10 days	6.5	−43	11.5	−37	17.7	12	29	−33	45	18	0.60	11	55	49	61	33	4.4	300	9.3	2
12 days	6.4	−44	12.3	−32	19.2	22	32	−26	50	32	0.64	19	—	—	—	—	4.0	264	10.9	20
14 days	6.7	−42	12.2	−33	18.2	15	30	−30	45	18	0.64	19	55	49	60	31	5.0	354	13.5	48
16 days	6.6	−43	11.6	−36	17.6	11	30	−30	45	18	0.62	15	60	62	63	37	4.2	282	14.3	57
18 days	5.0	−57	10.4	−43	20.8	32	26	−40	51	34	0.62	15	65	76	68	48	3.3	200	14.0	54
Liver extract administered																				
19 days	4.9	−57	9.9	−45	20.2	28	—	—	—	—	—	—	—	—	—	—	4.3	291	—	—
21 days	5.5	−52	11.2	−38	20.4	29	27	−37	49	29	0.58	7	66	78	68	48	6.0	445	11.3	24
23 days	7.0	−39	11.9	−34	16.8	6	28	−35	39	3	0.58	7	66	78	67	46	4.0	264	8.2	−10
Iron therapy started (25)																				
25 days	6.0	−48	11.1	−39	18.5	17	28	−35	47	24	0.54	0	65	76	67	46	4.4	300	10.5	15
28 days	6.0	−48	11.6	−36	19.3	22	29	−33	48	26	0.54	0	65	76	67	46	3.1	182	7.7	−15
31 days	5.8	−50	11.7	−35	20.2	28	32	−26	55	45	—	—	—	—	—	—	1.4	27	7.9	−13
35 days	5.6	−51	11.6	−36	20.7	31	33	−23	59	55	—	—	—	—	—	—	1.2	9	—	—
40 days	7.5	−35	13.4	−26	17.8	13	38	−12	51	34	—	—	—	—	—	—	0.8	−27	—	—

data obtained. The normal faeces were free of urobilinogen and the relatively large amounts present following the treatment indicated that the icterus was not of an obstructive origin. The figures presented are arbitrary colorimetric units and do not represent absolute amounts.

TABLE IV

HORSE *B*, EXPT. II

Detection of urobilinogen in the faeces

Post-treatment periods	Colorimeter reading
Normal	0
18–24 hr.	90
3rd day	242
4th "	157
7th "	94
8th "	62
9th "	155
10th "	52
11th "	172
12th "	161
14th "	125
15th "	167
16th "	116
17th "	90
25th "	50
39th "	10

Direct Van den Bergh tests for uncombined blood bile pigments were made on the first three days after treatment. No immediate reactions occurred, thus there was no suggestion of hepatic dysfunction or even of a temporary inability of the polygonal cells to combine the pigment.

The continuation of the high indirect readings was noted as being important in consideration of the haemolysis.

Tests of the urine for bile pigments could not be made by colorimetric methods owing to interference from the thiazone dyes. Surface tension tests did not reveal significant amounts of bile.

Clinical effects on the urinary tract were definite and characterized by thirst, proportional increase in urine, frequent urination, and, between the second and fourth days, severe pain over the loins. There was a definite haematuria and a high protein content in the urine on the second day; the erythrocytes and casts were numerous and the trichloracetic acid test revealed masses of protein precipitate. The protein decreased suddenly on the fourth day, but numerous erythrocytes were present until the fifth day, when the clinical symptoms of urinary tract damage were no longer present.

On the 18th day after treatment the secondary anaemia had become quite severe and a trial intramuscular injection of 50 U.S.P. units of liver extract

TABLE V

HORSE *B*, EXPT. II

Excretion of phenothiazine and derivatives in faeces and urine

Faeces

Post-treatment periods, hr.	Dry weight, gm.	Phenothiazine		Oxidation products (as leucophenothiazone), gm.
		Gm.	Gm./hr.	
0 – 12	618	0.95	0.08	0.28
12 – 14½	660	0.56	0.22	0.20
14½ – 16½	285	0.68	0.34	0.24
16½ – 22	956	2.40	0.43	0.61
22 – 23½	406	1.75	1.17	0.34
23½ – 25½ / 25½ – 28	620	1.20	0.27	0.65
28 – 30	333	0.84	0.42	0.29
30 – 36	467	1.25	0.20	0.33
36 – 47	410	0.72	0.07	0.23
47 – 50	423	0.46	0.15	0.24
50 – 52	455	0.24	0.12	0.14
52 – 54½	316	0.10	0.04	0.06
		Total = 11.2 gm.		Total = 3.6 = 3.3 gm. phenothiazine

Urine

Post-treatment periods, hr.	Volume, ml.	Total leucophenothiazone		
		Per cent	Gm.	Mg./hr.
0 – 4	1240	0.038	0.48	0.12
4 – 7½	930	0.204	1.89	0.54
7½ – 23	2270	0.256	5.82	0.38
23 – 31½	1880	0.202	3.80	0.45
31½ – 36	2230	0.022	0.49	0.09
36 – 48	3180	0.025	0.79	0.07
48 – 72	4455	0.020	0.89	0.04
72 – 76½	740	0.026	0.19	0.04
76½ – 80	630	0.022	0.14	0.04
80 – 86	1050	0.009	0.10	0.02
86 – 95	2070	0.008	0.18	0.02
95 – 101½	1310	0.010	0.13	0.02
101½ – 103½	1000	0.005	0.05	0.03
103½ – 119	2400	0.002	0.05	0.01
119 – 127½	2080	0.002	0.05	0.01
127½ – 143	2330	0.0003	0.01	—
Washings	11,340	0.0008	0.09	—
			Total = 15.2 = 14.0 gm. phenothiazine	

NOTE: *Total recovery = 28.5 gm. phenothiazine (= 47.5% of dose).*

was administered. The effects on the blood were not marked, although some improvement in the erythrocyte and haemoglobin levels could be noted (Fig. 1). On the 25th day a course of four daily doses of 4 gm. of ferrous sulphate with 0.1 gm. of copper sulphate was instituted. This treatment had no noticeable effect upon the trends.

In this experiment the anthelmintic effect was complete; nearly all the worm parasites (20,350 Cylicostomes, 577 *Strongylus* spp., and 175 *Habronema* spp.) were passed in the faeces between the 24th and 48th hours after treatment. A further recovery of 22 *Strongylus* spp. was made during the third and fourth days.

The previous tests for ova had remained fairly constantly at 2000 eggs per gram of faeces for four weeks; these tests dropped to zero on the second post-treatment day and did not become positive again for eight weeks. Qualitative tests (sugar flotation) remained negative for 14 post-treatment days, after which the findings varied from one to three eggs per slide for the next 30 days.

Table V shows the amounts of phenothiazine and oxidation derivatives from the excreta during the six days following treatment. Again it will be noted that less than 50% recovery was attained.

Experiment III

Horse *A* was again used for a test on the effects of a smaller dose, 97 days after it had been dosed previously. After the normal blood levels over a period of 16 days were determined, a dose of 30 gm. of pure phenothiazine was administered in the feed. No clinical effects could be noted and the blood examinations revealed only an insignificant haemolysis. The haematological and chemical data are presented in Tables VI and VII.

The anthelmintic effect was complete as were the effects from larger doses. A pretreatment quantitative count of 1200 eggs per gram immediately fell to,

TABLE VI

HORSE *A*, EXPT. III

The blood values following a dose of 30 gm. of phenothiazine

—	Erythrocytes, millions/mm.^{3}	Haemoglobin, gm., %	Erythrocyte volume, %	Cell fragility, % NaCl	Bilirubin, mg., %
Pretreatment normals	7.5(σ=0.5)	13.1(σ=0.5)	37.3(σ=1.5)	0.59(σ=0.00)	0.3(σ=0.1)
Values after treatment. Days					
1	8.5	15.3	38	0.57	0.6
2	8.6	15.2	39	0.58	0.5
3	6.9	14.0	37	0.60	0.3
4	8.4	14.7	40	0.58	0.3
5	7.7	13.2	—	—	0.2
7	7.7	14.2	37	0.60	0.3
14	7.5	13.9	38	—	0.3

TABLE VII

HORSE *A*, EXPT. III

Excretion of phenothiazine and derivatives in faeces and urine

Faeces

Post-treatment periods, hr.	Dry weight, gm.	Phenothiazine		Oxidation products (as leucophenothiazone), gm.
		Gm.	Gm./hr.	
0 – 16	360	0.03	0.002	0.02
16 – 16½	520	0.09	0.180	0.17
16½ – 20	460	0.24	0.068	0.20
20 – 22	324	0.28	0.140	0.15
22 – 24	445	0.36	0.180	0.24
24 – 28½	1580	0.39	0.086	0.65
28½ – 37	1120	0.06	0.007	0.21
37 – 40½	2480	0.12	0.048	0.20
40½ – 49	1330	0.00	0.000	0.13
		Total = 1.57		Total = 1.97 = 1.83 gm. phenothiazine

Urine

Post-treatment periods, hr.	Volume, ml.	Total leucophenothiazone		
		Per cent	Gm.	Gm./hr.
0 – 7	750	0.056	0.42	0.06
7 – 18½	2450	0.065	1.59	0.14
18½ – 26	1100	0.132	1.45	0.19
26 – 42½	3340	0.055	1.84	0.11
42½ – 66½	4000	0.014	0.64	0.03
			Total = 5.94 = 5.50 gm. phenothiazine	

NOTE: *Total recovery = 8.90 gm. phenothiazine = 29.7% of dose.*

and remained for 40 days at, zero; 5061 strongyloid nematodes were recovered from the faeces during the first two post-treatment days.

Discussion

Experiment I confirmed the fact that a cythaemolytic icterus may be produced by a dose of 0.05 gm. of phenothiazine per pound of live weight in a horse and that the effect upon the blood may be prolonged. Failure to recover more than 41% of the dose through the intestinal and urinary tracts seemed to indicate either fixation of a large proportion of the dose in the body or excretion in some form which the methods could not detect. The deriva-

tive recovered from the urine was a conjugate, very similar to, if not identical with, the conjugate recovered from the urine of treated sheep (1).

Experiment II, with more detailed observations on blood changes, provides data on the nature of the anaemia and icterus. It is noted that the primary anaemia is brought about by haemolysis of the circulating erythrocytes, with a relatively small increase in fragility. It was noted during pretreatment observations that this horse had an unusually large number of erythrocytes (nearly 12,000,000/mm.^{3}) and that a large proportion was microcytes, the mean corpuscular volume being 37 as compared with 51 for horse *A*. According to C. P. Neser* regular exercise results in a marked increase in erythrocytes; horse *B* was in excellent physical condition and did not tire easily, even with violent exercise. Neser found that clinically healthy horses varied in erythrocyte numbers from 5.25 to 11.5 millions per mm.^{3}; he recorded a count of 12.5 millions per mm.^{3} in a highly trained racehorse. The upward trends of the mean corpuscular volume and mean haemoglobin content seem to indicate that the smaller erythrocytes (or microcytes) are more susceptible to the haemolysing agent; the partial recovery of the blood elements on the fifth day was accompanied by a reduction in the mean volume and mean haemoglobin. Again, in succeeding waves of haemolysis, the mean corpuscular volume and haemoglobin increased. Although tests of numbers of horses would, of course, be necessary for significance, this observation suggests that animals having a large proportion of microcytes (or low mean corpuscular volume) would be more susceptible to the haemolytic anaemia produced by phenothiazine therapy.

The recurring haemolysis, correlated with the lack of recovery of even half of the dose by the methods used, seemed to give an indication that the proportion of the drug not immediately excreted by the body at least in a recoverable form, continued to act as a haemolysing substance in the body tissues. Another possibility is that the spleen became overstimulated and destroyed erythrocytes by hyperactivity; the descriptions of splenomegaly by Errington (2), Hatcher (9), and others, lends some support to such a hypothesis. It is evident, however, that active erythropoiesis continued.

The relatively high levels of the bilirubinaemia, particularly following a fall in erythrocytes, the presence of faecal urobilinogen and the absence of a definite direct Van den Bergh reaction suggested haemolysis of circulating cells rather than dysfunction of the liver or haemopoietic organs. It is evident that there were severe pathological changes in the urinary system; this observation suggests that in horse *B*, changes similar to those described in detail by Errington (2) were present. Errington recorded haemoglobinuria but not haematuria, although he found erythrocytes in the tubules of damaged kidneys.

A series of experiments designed to show details of the mechanism of the haemolysis is in progress.

* *Neser, C. P. 9th and 10th Rept. Dir. Vet. Ed. Research, S. Africa, 481-558. 1924.*

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